JOURNAL OF

AGRICULTURAL

RESEARCH

VOLUME 35
JULY 1–DECEMBER 15, 1927

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

All correspondence regarding articles from State experiment stations should be addressed to R. W. Thatcher, Agricultural College, Amherst, Mass.

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Published on the first and fifteenth of each month. This volume will contain twelve numbers and the Contents and Index.

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ERRATA AND AUTHORS' EMENDATIONS

Page 143, Table 4, last column, "5.83" should be "5.30."

Page 177, fourth line from bottom, "1.313" and "1.537" should be "1,313" and "1,537."

Page 288, lines 3 and 4, should be interchanged.

Page 445, third line from bottom, footnote numeral 6 should be inside parenthesis, thus: "*P myriotylum* Drechsler, ined.[6].)"

Page 451, in heading, "Organism" should be "Virus"

Page 564, first line of legend of Figure 1, insert after "branch" "(the lowest only)."

Page 657, line 12, "coordinated" should be "correlated."

Page 729, Table 1, heading of the fifth column should be "Density, 20° C "

Page 781, lines 9 and 23, "Hcl" should be "HCl "

Page 798, Table 1, seventh column, unit under "Time of concentration," "Minimum" should be "Minutes."

Page 801, near middle of page, formula should be "$Q = (3.83 - 0.07L)\ Lh^{(1.52-0.01L)}$," that is, the quantity in the second parenthesis is an exponent.

Page 806, Table 5, last column, references to Figures 9, 10, 11, and 12 should be to Figures 11, 12, 13, and 14, respectively.

Page 913, Figure 6 is reversed

Page 930, the table and the paragraph printed in small type belong to the footnote at bottom of page

Page 1026, line 24 (end of par. 3), "pl. 2" should be "pl. 4."

Page 1027, eighteenth line from bottom, "Plate 2" should be "Plate 4 "

Page 1031, line 17 "(pl. 3, A, B, C)," should be "(pl. 1, C–F)."

Page 1039, line 8, "readily" should be "really."

Page 1041, citation (25), delete "[Original not seen. Abstract in Rev. Appl. Mycol. 4. 707–708. 1925]."

Page 1043, line 8, "20 per cent" should be "30 per cent."

Page 1054, line 6, "indelible" should be "inedible."

Page 1094, fifth line from bottom, "national" should be "natural."

Page 1173, Table 33, "Tumbleweed," "Pigweed," "Russian thistle," "Lamb's quarters," and "Polygonum" should have a separate heading, "Weeds."

Page 1173, "Table 33, first column, under "Wheat: C. I. 4131," "S. P. I. 3715" should be "S. P. I. 37159."

DEVELOPMENT OF FLOWERS AND SEED IN THE SUGAR BEET [1]

By ERNST ARTSCHWAGER

Associate Pathologist, Office of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In comparison with its anatomy, which has been the subject of extensive research, the flower development of the sugar beet has received scant attention. Practically all previous work was unmediated, and we look in vain for a consecutive account of the development of the flower and fruit. Very recently, however, after the work recorded in this paper had been completed and submitted for publication, there appeared a paper by Dudok van Heel (2) [2] in which the cytology and embryogeny of the sugar beet had been worked out in much detail with all important features illustrated by means of semidiagrammatic drawings. This paper, however, complete in all other respects, does not take account of the general development of the flower and the relationship of the leaf and floral traces.

MATERIALS AND METHODS

The material used in the present study was grown at Fort Collins and Fort Lewis, Colo. The different strains had been selected and grown by W. W. Tracy for a number of years and possessed a great uniformity in foliage and root characters. Since the strains were grown separately in well-isolated localities they seemed suited for this work, especially since pure lines are unknown and commercial stock is a hopeless mixture. Material was fixed in Carnoy's fluid—three parts of absolute alcohol and one part of acetic acid. Other material was fixed in Allen's modification of Bouin's solution which is suitable for counting chromosomes in certain stages. The material was embedded in paraffin, cut from 5 to 15 μ thick, and stained with Haidenhain's iron-alum haematoxylin.

DEVELOPMENT OF THE SEED BUSH

The cultivated beet is a biennial and develops its seed during the second year. When planted out the second season the plants first produce a rosette of leaves like those of the first year, but after about six weeks of growth the newly formed leaves become progressively smaller and finally the apical portion begins to elongate,

Journal of Agricultural Research,
Washington, D. C.

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thus initiating the development of the floral axis. The latter grows with great rapidity, but the leaves which develop on this shoot are much smaller than the rosette leaves and more widely spaced. In the axils of the leaves shoots arise which also grow rapidly and give rise to branches of the third order. The mature inflorescence or "seed bush" is composed of large, paniculate, more or less open, spikes bearing the flowers and later the seed.

The sessile flowers are usually in clusters of two or three (fig. 1, C) which are attached to the inflorescence axis or secondary branches of the latter. The small, greenish flowers (fig. 1, A) are perfect, the calyx is 5-parted and incurved, adhering to the base of the ovary and becoming hard in the fruit. There are five stamens inserted at the base of the calyx lobes. The stamens are introrse, bilocular, and open by longitudinal fissures. The 3-carpellary ovary is sunk in a fleshy disk inclosing a campylotropous ovule which is attached laterally to the ovary wall by a short funiculus. The style is very short, terminating in a 3-lobed stigma which persists in the fruit. The latter is an aggregate, formed by the cohesion of two or more flowers grown together at their bases and forming a hard and irregular dry body, the so-called "seed ball."

An illustrated account of the development of the flower of *Beta maritima* is given by Payer (*7, p. 310–311, pl. 66*), and with it the description of the sugar-beet flower, as given below, tallies. The rudiments of the young flowers appear close behind the growing point of branches of the inflorescence axis. Each rudiment is borne in the axil of a large bract and is accompanied by two lateral bracts each of which has in its axil a flower. The flower primodium forms a small protuberance on which are soon differentiated five narrow ridges, the beginnings of the sepals. Next there appears within this circle a whorl of papillae, the stamen rudiments each opposite a calyx lobe. Finally three more ridges develop at first separately but later joining to inclose a more or less compressed roundish cavity in which a single ovule develops.

Once initiated, the primordia of the different floral parts develop very rapidly. The young stamens become stalked early and attain in cross section the characteristic quadrilocular form. The anther is at first a homogeneous mass of tissue covered by an epidermis. As soon as it becomes faintly 4-lobed in cross section the cells next to the epidermis begin to elongate radially and divide periclinally, forming two layers of narrow cells which by their size and staining reaction appear distinctly set off from the central tissue which forms the archesporium. These two layers of cells together with the epidermis constitute the anther wall. The cells of the middle layer, in their subsequent development, enlarge rapidly and finally develop thickening bands. This constitutes the endothecium and forms a continuous mantle except in the region where the anther later dehisces. The inner layer is in places more than one cell wide. The cells remain small and as the anther enlarges they become stretched tangentially, but they do not become obliterated before the endothecium is fully developed and the pollen practically mature.

The ovule arises near the base of the carpels. It is seen first as a slight protuberance which enlarges to form the nucellus. In its subsequent development the young ovule becomes distinct from the

surface of the carpel by a stalklike base or funiculus. Very early there appears at the base of the nucellus an annular outgrowth followed soon by a second one of a similar nature. The first outgrowth
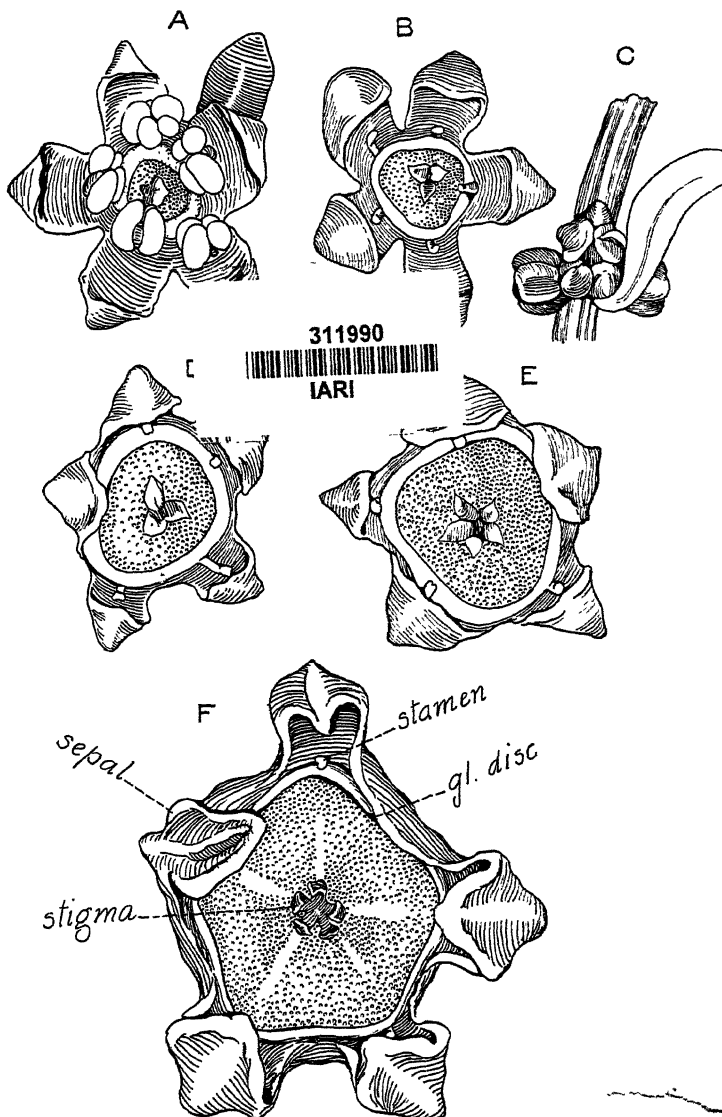
FIG. 1.—A, single mature flower viewed from above; B, flower with stamens already dropped; C, cluster of flowers attached to branch of inflorescence axis and subtended by a bract; D, flower after fertilization; E, flower with young embryo; F, flower with mature embryo. All × 10.25

develops into the inner, the second into the outer integument. The young ovule is straight, but with the appearance of the integuments it curves and becomes campylotropous. Finally, the ovule twists about its own funiculus and comes to lie horizontally in the cavity of

the ovary. This change in position as pointed out by Meunier (6) is necessitated by the form of the ovary cavity and made possible by the structure of the funiculus.

ANATOMY OF THE FLORAL AXIS

A transverse section through the young floral axis shows a circle of vascular bundles limited on the inside by a well-developed pith, on the outside by the epidermis and cortex (fig. 2, B). The pith is homogeneous, but the cortex possesses ridges of collenchymatous tissue near the periphery. The epidermis is one cell thick and abuts directly on the collenchyma or cortex.

The vascular tissue follows naturally a longitudinal course in the axis and its arrangement is closely related to the phyllotaxis, which is, however, often subject to variation. The normal arrangement is alternate with a divergence of two-fifths; that is, leaf number six stands directly over leaf number one after two turns of the spiral. Frequently the lower leaves of the inflorescence appear in pairs whereby a pseudo-opposite arrangement is effected. In traversing the stem vertically the bundles remain at approximately the same distance from the center except in the node, where certain bundles approach and leave the periphery alternately and acquire a somewhat undulate course.

The vascular bundles as seen in cross section are of different magnitude. Occupying the corners of the irregular polygon. (fig. 2, B) are five large cauline bundles alternating with an equal number of strands, usually somewhat smaller in size, which represent the median traces of the first five leaves. Flanking the sides of the cauline bundles are small strands of vascular tissue which represent the lateral traces. Plate 1, G, and Figure 3 show the course and the origin of the different traces and their relation to each other and to the stem. A study of the petiole of a very young leaf shows that there are three foliar traces, a large central one and two smaller ones occupying the flanks of a flattened semicircle. As a rule, however, there are many more bundles even in the youngest petioles, so that it is very difficult to follow the course of these various strands and to establish their origin. But neglecting the variations that are commonly met with, the derivation of the traces accords with the following plan: Each new median trace is derived from a left cauline bundle (viewed from the stem apex) a little distance above the node, it ascends without fusion or forking for five internodes and passes into the leaf without branching. The left lateral trace is derived from the cauline bundle next it, passes through three internodes, and usually branches several times before entering the leaf. The right

Plate 1

FIG. 2.—A, partial cross section of floral axis: *e*, epidermis; *c*, collenchyma; *cort*, cortex; *en*, endodermis; *s. c.*, secondary cambium; *ph*, phloem; *p. c.*, primary cambium; *x*, xylem; *p*, pith. × 144. B, cross section through young floral axis; *c*, cauline bundles; *m*, median trace. × 24.5

lateral trace is also derived from its adjacent cauline bundle. It
passes through only two internodes, but otherwise conforms to the
left lateral trace. The length of the lateral trace is, however, subject
to variation since it often remains a part of the cauline bundles for
a longer or shorter portion of their ascent.

In each node, then, the cauline bundles flanking the oldest median
trace undergo the following changes: A little distance below, or
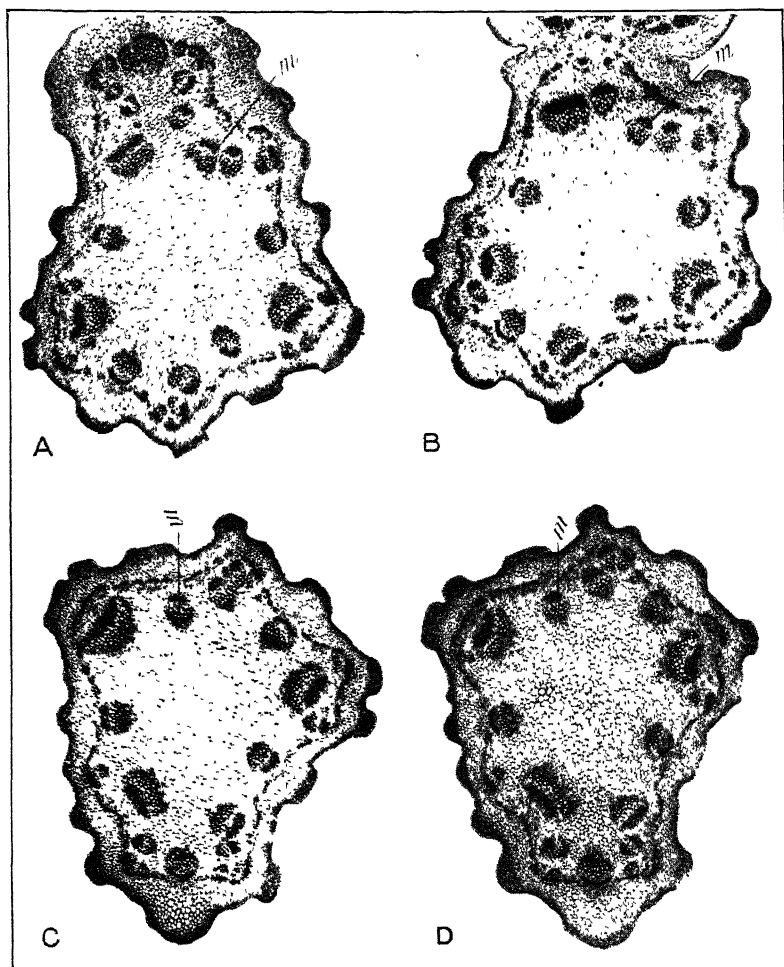
FIG. 3.—A Cross section through floral axis just below a node; B, the same but taken at the inser-
tion point of the leaf, C, the same but taken just below the node of the next higher leaf; D, the same
but taken a little higher up the stem. All × 14.25
The new median trace is designated with *m*, note its position in the different cuts

sometimes above the insertion of the leaf each cauline bundle gives
off a small branch which becomes a new lateral trace; a little higher
up they approach each other and may even temporarily fuse. Upon
separation, the left cauline bundle branches once more and the new
strand which is segregated becomes a new median trace, as shown in

Plate 1. The vascular supply of the axillary members is also derived from the cauline bundles a short distance below the node.

Fron (*1*) describes and pictures the origin and course of the vascular bundles in the floral axis of *Beta cicla* and states that the course of the bundles in *Beta vulgaris* is similar. According to Fron, the median trace splits at a distance of three internodes below its point of entrance, and the two resulting bundles pass into the pith for a short distance in their downward course and after traversing two or three more internodes attach themselves to their adjacent cauline bundles just below the node at which the latter enter the stem.

Although the vascular skeleton of the floral axis of the sugar beet shows a great deal of variation, the differences are not fundamental and never of a type which would conform to Fron's account.

The traces to the various floral organs are joined to the peduncle by a short receptacular stele in which the vascular tissue forms a somewhat irregular central core instead of the usual ring of separate bundles. With the broadening of the receptacle five distinct groups of vascular tissue separate off and diverge to occupy a position in the peripheral region of the cortex, while the remaining tissue of the core unites into a single bundle which becomes continuous with the vascular supply of the ovule. (Fig. 4, A, B.) Simultaneous and progressive changes are now seen in all parts. The periphery of the receptacle becomes distinctly 5-lobed and into these lobes vascular strands pass which have separated from the five cortical bundles (fig. 4, C); these bundles later branch again to form the lateral traces of the calyx lobes. From the central portion of the receptacle the ovary is now formed. The surrounding tissue constitutes the three fused carpels to the side of one of which a single ovule is attached by a short funiculus. Fundamentally, each carpel is supplied with three vascular strands—a central bundle and two marginal ones. In the flower of the sugar beet the marginal traces have been suppressed so that each carpel is supplied with only a single trace. These traces are derived from the cortical bundles in such a manner that two of them constitute direct branches of two cortical bundles lying opposite them, while the third carpellary trace is formed as a fusion bundle of two vascular strands derived from the adjacent cortical bundles. Occasionally these two bundles remain separate so that a cross section through the upper part of the pistil shows four bundles instead of three. Ultimately, these bundles fuse or one of them becomes reduced and ends blindly in the tissue of the carpel. What remains of the five cortical bundles after the sepals and carpels have been supplied, passes out into the stamens, each of which has a single trace (fig. 4, D). The course of the vascular supply in the flower and the mode of origin and course of the traces supplying the different floral organs is illustrated in the semidiagrammatic sketch (fig. 4, A to D). Plate 1, F, shows a still unopened flower with its vascular supply in median longitudinal section.

SECONDARY GROWTH IN THE FLORAL AXIS

After the formation of the primary bundles in which a normal cambium functions for a certain length of time, an interfascicular cambium differentiates. The segments of this cambium are formed in such a way that one flank of a segment becomes continuous with
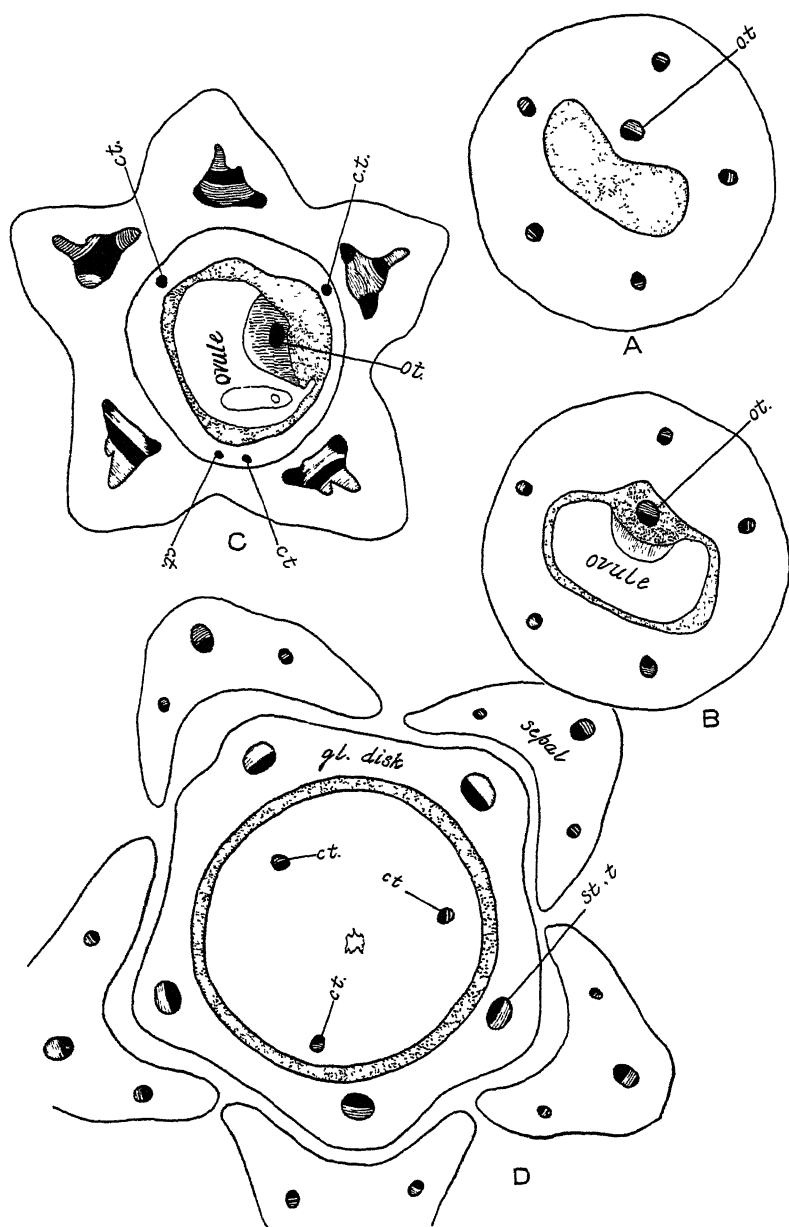
FIG. 4.—Diagrammatic drawings of a series of sections through a flower to illustrate the origin and course of the vascular supply: A, section through base of receptacle. There are present five peripheral bundles and a sixth one *o. t.* in close proximity to the base of the ovary cavity; B, section taken at a slightly higher level. Bundle *o. t.* is now seen in the cavity of the ovary; it forms the vascular supply of the single ovule; C, section taken at a level where the sepals are differentiating. The peripheral bundles are branching. Four strands designated *c. t.* have separated to form the vascular supply of the three carpels; D, section through the upper part of the flower. The sepals are now distinct. The filaments of the stamens are still a part of the glandular disk region. Each stamen is supplied with but a single trace

the normal cambium of the bundle while the other end arches around the phloem of another bundle, as shown in Figure 2, A. This method of unilateral connection was first noted by Hérail (*4*) and later substantiated by Fron (*1*) for other Chenopodiaceae.

As the activity of the normal cambium ceases, a new cambium arises in the pericycle so that at this stage the cambium ring is in part normal, in part pericyclic. Later it becomes entirely pericyclic.

Cell division in the new cambium is at first centripetal whereby xylem and parenchyma are formed. After a limited period of centripetal growth the cambium forms phloem and parenchyma centrifugally. The segments of cambium later mature completely into vascular tissue and parenchyma, whereby the ring of cambium is again broken. Before this occurs new segments of cambium are differentiated in the parenchyma on the outer phase of the phloem, and these new segments unite with the older cambium on either side. The new cambium undergoes the usual reciprocal divisions producing xylem and phloem. This process is repeated as long as active growth in the stem continues.

MICROSPOROGENESIS

The cells of the archesporial tissue of the young anther develop rapidly to the mother-cell stage. At the beginning of meiosis these cells show a net work of dense granular cytoplasm; the nuclei are large and contain a single nucleolus lying in a more or less central position. The chromatin is in the nature of fine granules distributed over a delicate linen net. Fine threads sometimes extend to the nucleolus which otherwise appears as if suspended in a hyaline vacuole. The chromatin granules are of uniform size and tend to aggregate along the nuclear membrane. Gradually the linen net becomes more permanent, the number of threads increases, and the threads themselves become thicker and the granules larger (fig. 5, A). The nucleolus shows frequently an appearance of budding. The protuberances separate off and diffuse out into the reticulum, becoming identical with the chromatin there present.

Without showing distinct pairing, the chromatin threads now contract toward one side of the nucleus. During this period of maximum contraction (fig. 5, B) the synaptic mass is very dense and the association of the chromatin threads becomes very intimate. The passing out of synizesis is first manifested by loops of free spireme being thrown into the nuclear cavity (fig. 5, C). These continue to spread and soon the entire chromatin cord is distributed through the nuclear cavity forming the well-known hollow spireme (fig. 5, D). The larger portion of the spireme is arranged along the nuclear membrane, but it also traverses the cavity in various directions. The appearance of the nucleus after synizesis differs from the preceding condition since the chromatic elements pass into synapsis as distinct bodies and emerge as a uniform filament.

The synaptic stages are somewhat prolonged and the loosening and the formation of the spireme also take place gradually so that the persistence of the "Klumpen" stage, as also shown by Dudok van Heel (*2*), is only apparent, and what appears to be a *Klumpen* when viewed under high power is only an intermediate stage; that is, the
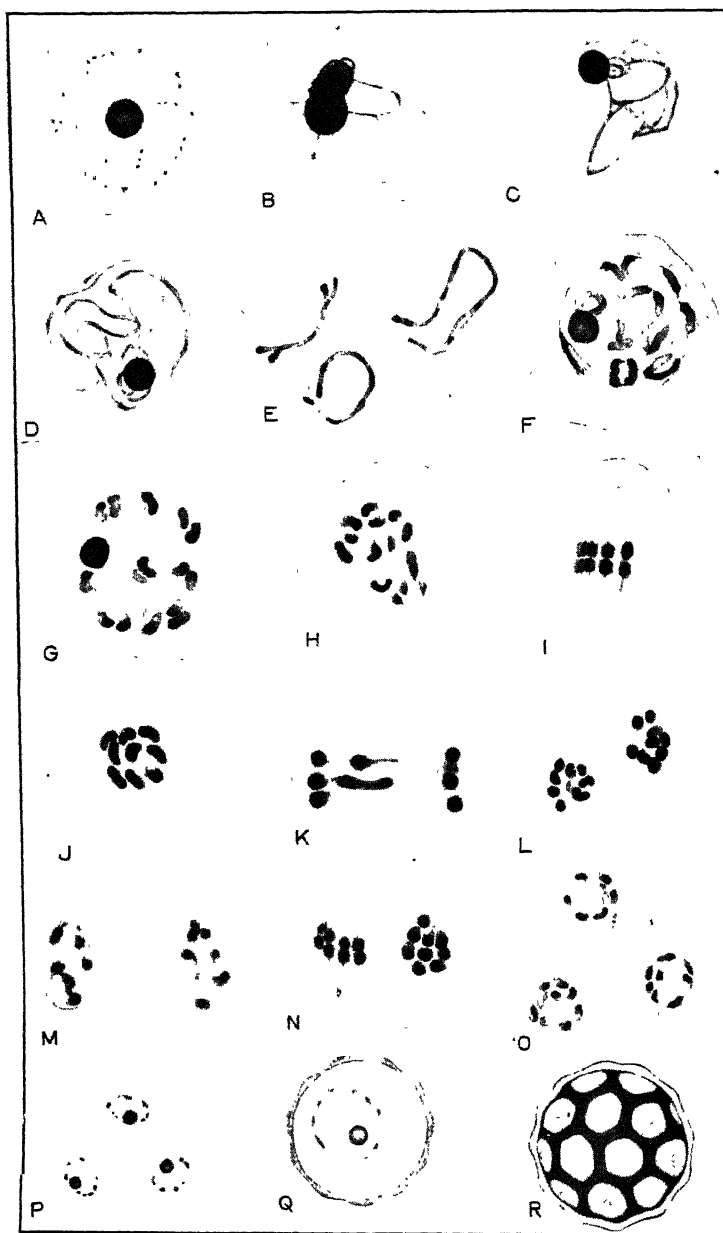
Fig. 5.—Meiosis in microspore development: A, early prophase; B, synizesis; C, postsynizesis; D, hollow spireme; E, segmentation of spireme to form the chromosomes; F, early diakinesis; G, late diakinesis; H, multipolar spindle; I, metaphase; J, polar view of metaphase; K, anaphasic separation; L, late anaphase; M, telophase, N, homotypic spindles, O, homotypic telophase, P, tetrad formation; Q, young pollen grain; R, mature pollen grain, surface view

Klumpen in the process of being formed or in the initial stage of its loosening. However, the subsequent stages as well as the homotypic division are passed through very rapidly.

Without going into second contraction the previously paired spireme threads now segment into pieces of various lengths which are twisted hither and thither in the nuclear cavity. The segments commonly have the form of a U (fig. 5, E), but they may be of various shape. The chromosomes or gemini now undergo the usual thickening and shortening and eventually attain a compact form. The various pairs are at first distributed throughout the nuclear cavity (fig. 5, F), but gradually they become limited to the nuclear membrane (fig. 5, G). The nucleolus at this stage is slightly reduced in size and occupies an eccentric position which makes the counting of the chromosomes more difficult. The appearance of the chromosomes in diakinesis does not deviate from the normal condition. There are commonly nine pairs present with no unpaired univalents, but owing to the smallness of the chromosomes it is not always possible to determine the latter point with certainty.

The arrangement of the chromosomes in diakinesis is quickly followed by the disappearance of the nuclear membrane and the nucleolus. A multipolar spindle is differentiated (fig. 5, H), but as the chromosomes approach the equator the spindle becomes distinctly bipolar (fig. 5, I). The disappearance of the nuclear membrane and the formation of the multipolar spindle are so quickly accomplished that one rarely sees these stages in a preparation. Cross sections through an anther often show the chromosomes of one locule of the theca in diakinesis, while in the other locule they are already arranged on the equator. Since in the sugar beet any given meiotic stage occurs simultaneously throughout a locule, it becomes necessary to study cross sections in order to determine the proper sequence of the different stages and their relative duration; this is especially necessary when the stages are passed through rapidly.

At the equatorial plate the chromosome are seen as thick, more or less roundish, lumps which in polar view appear distributed over a more or less circular space (fig. 5, J). The two members of each bivalent now separate in metakinesis, one going to each pole. On their way to the pole certain chromosomes may show lagging (fig. 5, K), but on the whole the anaphasic separation is quite normal. After a very brief interkinesis (fig. 5, M), the chromosomes of each daughter nucleus pass on to the homotypic spindle (fig. 5, N). The two spindles may lie parallel or at right angles to each other. On the spindle the chromosome halves separate and thus the longitudinal split is completed.

The nuclear plate in the heterotypic anaphase contains nine chromosomes. These are at this stage almost spherical and as a rule lie well apart. Winge (*10, p. 178*) and Dukok van Heel (*2*) also found nine chromosomes to be characteristic for Beta and for other members of the Chenopodiaceae. Matthysen's (*5*) finding of eight chromosomes in the cultivated garden beet is at variance with these figures and in the light of the present account is open to question. However, the writer also was able to count but eight chromosomes

in some of his sugar-beet material, but such small irregularities should be of no significance.

After the second division (fig. 5, O, P), each of the young microspores becomes invested by a wall which at first is thin and delicate. As the spore increases in size, the wall becomes differentiated into two layers (fig. 5, Q). The outer layer is thick and sculptured and contains numerous thin spots for the exit of the pollen tube (fig. 5, R). The microspores become free from one another at maturity, forming a powdery mass which, when the anther dehisces, is distributed by wind and insects.

The tapetal layer in the developing anther is separated from the primary sporogenous tissue after the three layers of the anther wall have become distinct. Although showing the same staining reaction, the young tapetal cells can be distinguished from the sporogenous tissue not only by their peripheral location but also by the shape and size of the individual cells. When the sporogenous cells enter the mother-cell stage, the cells of the tapetum begin to enlarge radially, but axially their length remains about the same. In stained sections they appear darker than the sporogenous cells and this feature persists until they disintegrate. At about the time the sporogenous cells enter synapsis the tapetal cells become binucleated. By this time the cells have increased their radial diameter to such an extent that the anticlinal walls are more than double the length of the periclinal ones (fig. 6, A, and fig. 7, A). However, not all of the cells are narrow; some of them enlarge tangentially as well as radially and appear almost square in cross section, while still others are polygonal or otherwise irregular. The nuclei of the tapetal cells are conspicuous by the large quantity of dark-staining granules. There is commonly only one nucleolus, but occasionally some of the chromatin granules are so large as to simulate secondary nucleoli. The cytoplasm of the cells is also very dense and takes a darker stain than the cytoplasm of the sporogenous cells. At the time of the appearance of the tetrads the tapetum cells show signs of degeneration (fig. 7, B). They gradually lose their content which is being absorbed by the developing pollen. The nuclei, however, retain their granular appearance for a long time, but finally they too become disorganized. Their walls remain intact until the pollen is mature, but they collapse prior to the dehiscence of the anther and the shedding of the pollen (fig. 6, B).

It is evident from the foregoing description that the nuclear phenomena in microsporogenesis of the sugar beet conform generally to the process in other plants (*8*). The early prophase stages appear complex and have been described differently by Matthysen (*5*), who studied meiosis in the garden beet. But since details in cell division may be studied profitably only in organisms possessing large nuclei, little is gained by attempting it with structures so small as the chromosomes of the sugar beet. However, since genetical researches carried on in connection with breeding problems require for a background a knowledge of chromosome behavior in post-synizesis rather than an exact interpretation of the early prophase stages, the object of this investigation has been realized.

<div style="text-align:center">MACROSPOROGENESIS</div>

The archesporium develops from a single hypodermal cell of the nucellus. The epidermal cells divide several times tangentially whereby the sporogenous cell of the megasporocyte becomes embedded in

FIG 6.—A, partial cross section through tapetum at a stage when the sporogenous tissue is in late prophase; B, section through mature anther. The pollen is fully formed while the cells of the tapetum have practically degenerated   × 820

nucellar tissue (fig. 8, A). At the close of the first meiotic division in the megasporocyte a wall is formed between the two nuclei, but the two daughter cells divide rapidly again to form the four megaspores (fig. 8, B). The outer three megaspores degenerate while the inner

one forms the embryo sac. The young embryo sac is straight, but soon the nucellar cells at the chalazal end begin to degenerate and as the ovule continues to curve disintegration of the nucellar tissue also proceeds, until a more or less horseshoe-shaped cavity results. The curvature of the embryo sac is hastened by cell division in the concave part of the nucellus (fig. 9, A), and through these divisions a tissue is produced which later becomes filled with starch and which forms the perisperm of the mature seed. The cavity of the embryo sac is lined with broken cell remains. At the basal end of the mature sac an accumulation of protoplasm is commonly found with numerous embedded nuclei. The nucleus of the functional megaspore initiates the usual series of three equational divisions with the resulting nuclei lying free in the embryo sac. The egg and the two synergids
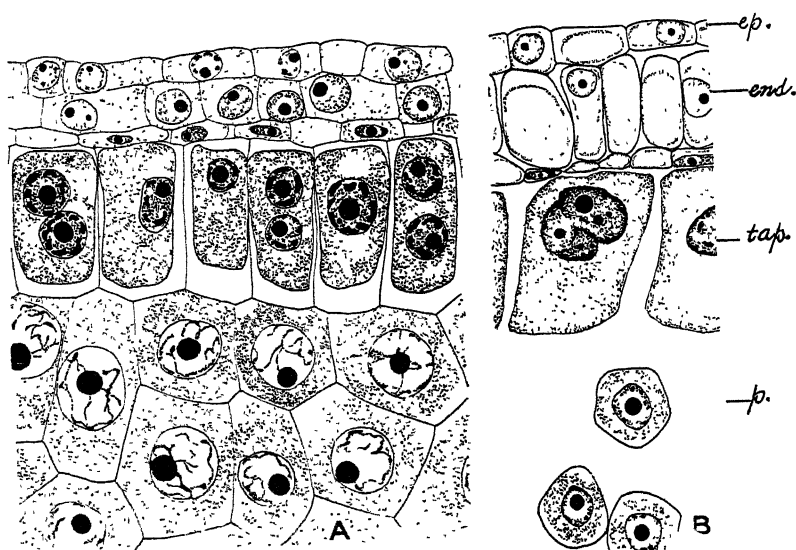
FIG. 7.—A, cross section through wall layer, tapetum, and peripheral sporogenous tissue of a young anther; B, an older stage. Note that the second wall layer has greatly enlarged while the cells of the third layer have almost completely degenerated. The tapetal cells are at this stage already in the incipient stages of degeneration

are located in the micropylar end of the sac. The two synergids partly surround the larger egg which bears its nucleus in the end toward the center of the sac (fig. 10, A). The two polar nuclei fuse some time before fertilization. The resulting fusion nucleus is very large with a well-marked nucleolus and a dense reticulum. The antipodal cells which are found in the chalazal end of the sac are well developed. They begin to degenerate before the embryo sac is mature, but often only after the egg is fertilized.

FERTILIZATION AND EMBRYOLOGY

The mature beet flowers begin to open in the morning; the sepals expand horizontally, leaving the stamens exposed and in an upright position. If the day is warm and sunny the anthers dehisce in the forenoon and the pollen is carried by wind and insects to the receptive stigmas of older flowers. Shaw (*9*) considered thrips to be the most
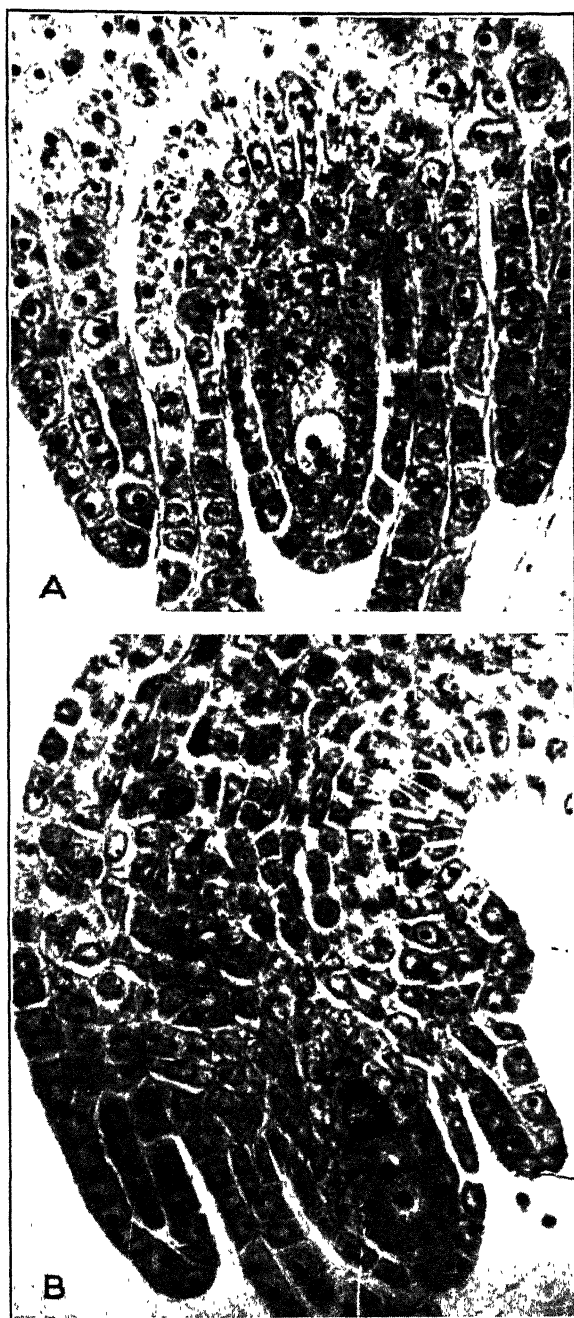
FIG. 8.—A, longitudinal section through young ovule with megaspore mother cell, × 700; B, longitudinal section through a megaspore tetrad. The innermost cell becomes the embryo sac, × 700
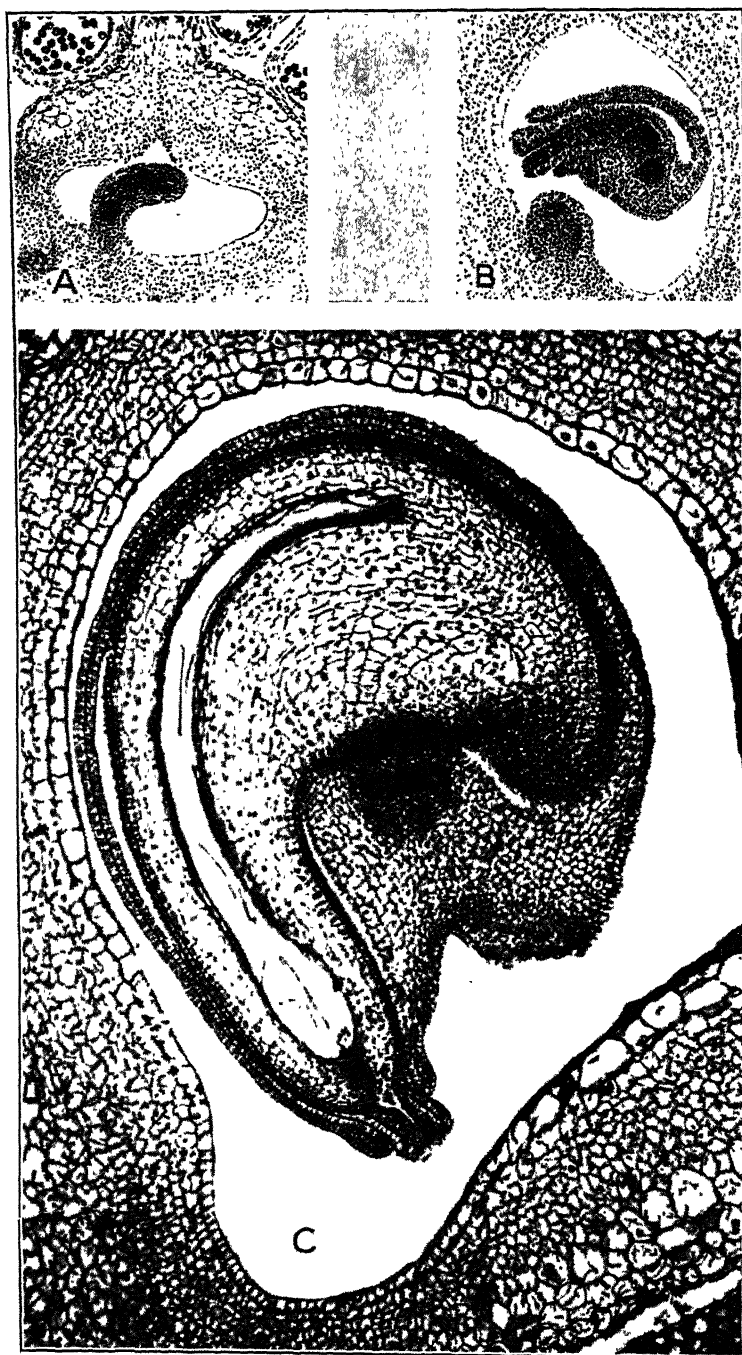
FIG. 9.—A, longitudinal section through ovary with young ovule, × 70; B, cross section through ovary showing the young ovule in longitudinal section, × 94; C, longitudinal section through ovule at an age when the egg is fertilized, × 60

active pollenizers, a fact that well agrees with the observations of
the writer, who invariably found thrips present in flowers examined
by him.   In countries where thrips do not occur, the wind, as sug-
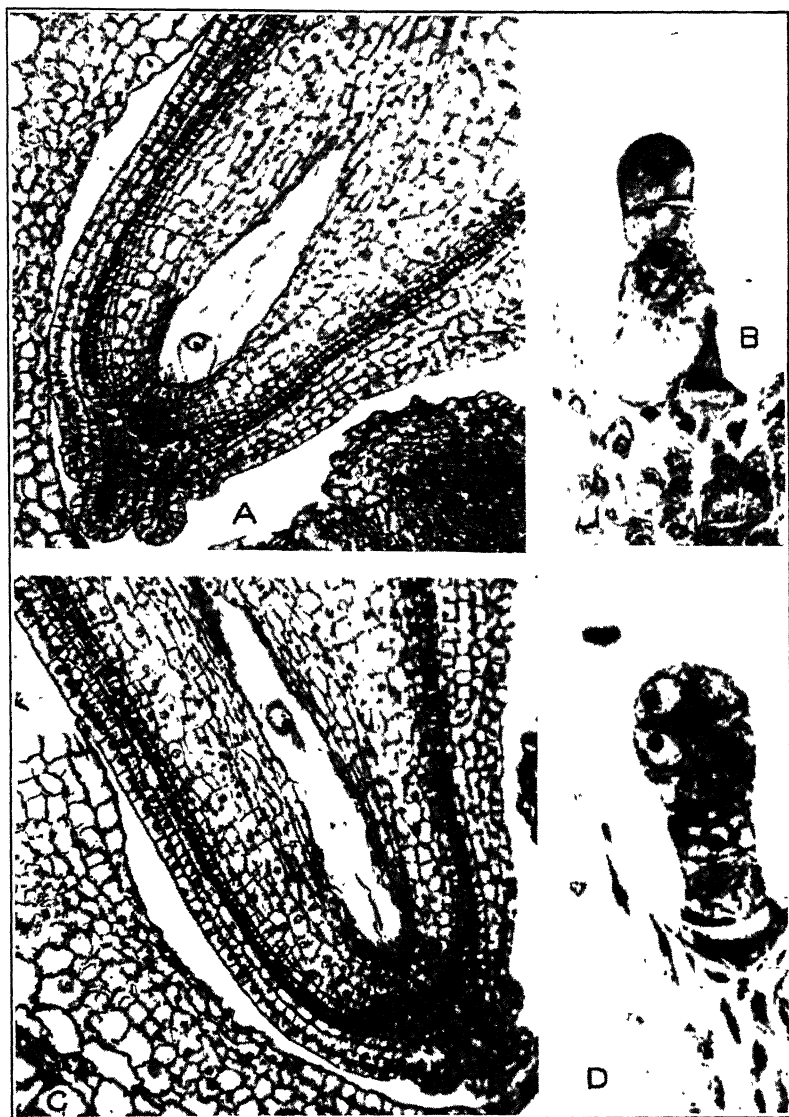
FIG. 10.—A, longitudinal section through micropylar end of ovule, showing the egg nucleus in the
embryo sac, × 175; B, 2-celled embryo, × 654, C, different section of the same material as A show-
ing the fused polar nucleus, × 175 D, 6-celled embryo, × 654

gested by Dudok van Heel (*2*), is probably the most effective agent
in the dispersal of pollen.

    At the time of the opening of the flower the stigmatic lobes are still
closed (fig. 11, A).   They begin to open very gradually in the after-

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noon but may not fully expand until the next day or the following one (fig. 11, B). One frequently observes flowers with anthers already shrivelled but completely closed stigmatic lobes; on the other hand, the shedding of the pollen and the opening of the stigma lobes may occur simultaneously, though such cases are very rare.

The embryo sac at the time of fertilization (fig. 12, A) has only two large nuclei; the egg and the fused polars. The synergids and the antipodals are smaller and at that time are often already in the process of degeneration. When the pollen tube reaches the "egg apparatus" through the micropyle it passes between the synergids and applies itself closely to the egg. The actual discharge of the male nuclei, however, has not been observed. Taking the presence of the pollen tube as evidence that fertilization has occurred, it appears that fertilization takes place at a time when the stigmatic lobes have fully expanded; that is, a day or more after the pollen is shed and that, therefore, normally self-fertilization does not occur. The fertilized egg, immediately or after a brief period of rest, begins to divide to form the embryo. Dudok van Heel (2) is of the opinion that the pollen often germinates while the stigmatic lobes are still closed and that fertilization takes place immediately; the fertilized egg, however, rests for several days before it divides again.

The ovule at the time of fertilization is hardly more than 1 millimeter in diameter, but the embryo sac is well developed and digestion of the nucellar tissue proceeds rapidly until the end of the sac arrives in the proximity of the chalaza where it may enlarge somewhat and show an accumulation of nuclei and cytoplasm. After fertilization has occurred, the ovule grows rapidly (fig. 1, B, C, D, E) and attains practically the size of the mature seed before the embryo is fully developed. During this growth of the ovule the sepals of the flower also enlarge and commonly curve back again to a position approximating that of the opening of the flower.

After fertilization the oospore divides horizontally (fig. 12, C) giving rise to two cells (fig. 12, D, fig. 10, B). Soon the lower of the two cells divides again transversely and this is followed by a division of the upper cell. Hereby a tetrad of superimposed cells is formed of which the upper three, to all appearances, give rise to the embryo proper while the fourth develops the suspensor. The latter is filamentous but more than one cell wide.

After the embryo has grown to the 4 or 6 celled stage, the primary endosperm nucleus, formed by the union of the fused polars with the second male nucleus, begins to divide and the nuclei migrate after a manner described by Hegelmaier (3) and Dudok van Heel (2) toward the periphery, where they become embedded in the cytoplasm and continue to divide (fig. 13, A). At the micropylar end, especially in the vicinity of the lower suspensor cells more divisions occur and the cytoplasm appears more dense. After a period of free nuclear division the endosperm nuclei become invested by walls and gradually the entire cavity of the embryo sac is filled with a delicate tissue (fig. 13, B) which, however, is again dissolved and the content absorbed by the growing embryo with the exception of a single layer of cells surrounding the radicle. This layer becomes distinct from the rest of the endosperm tissue only after the embryo is partly grown (fig. 14). Its cells are small, very regular, and contain a dense granular content.
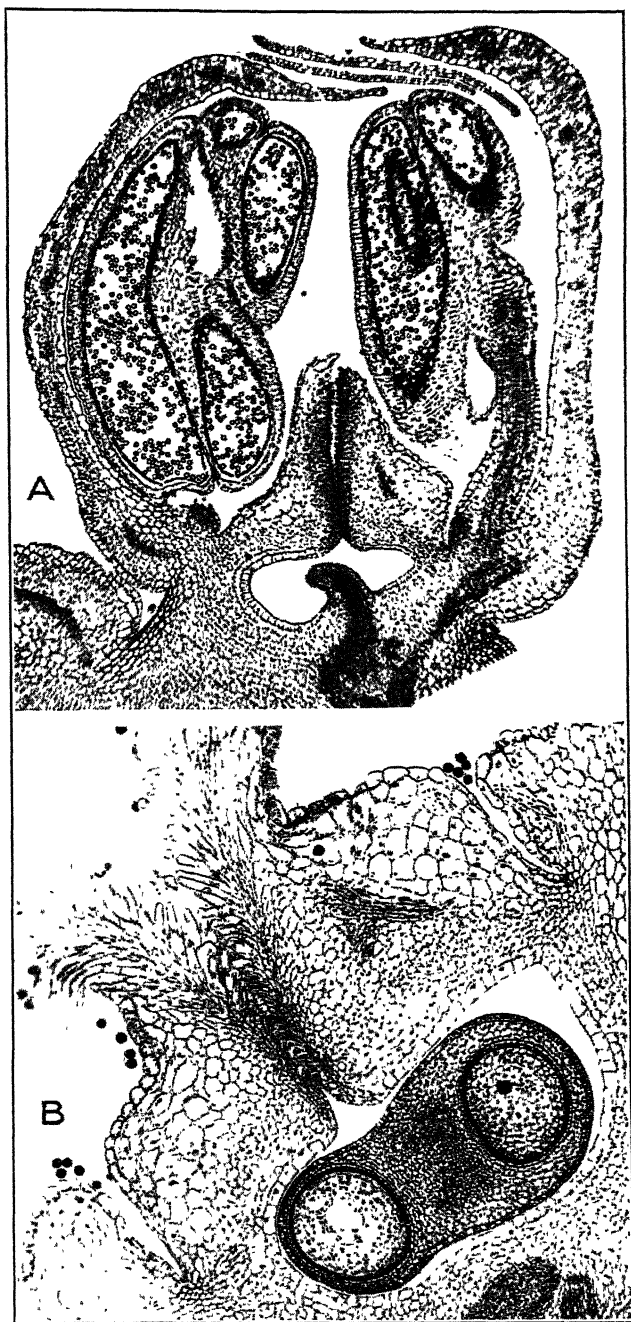
FIG. 11.—A, longitudinal section through a young flower, ✕ 58, B, longitudinal section through an older flower. The ovule is seen in cross section. The stigmatic lobes have spread apart ready to receive the pollen which is seen adhering to the outer surface of the stigma. ✕ 74
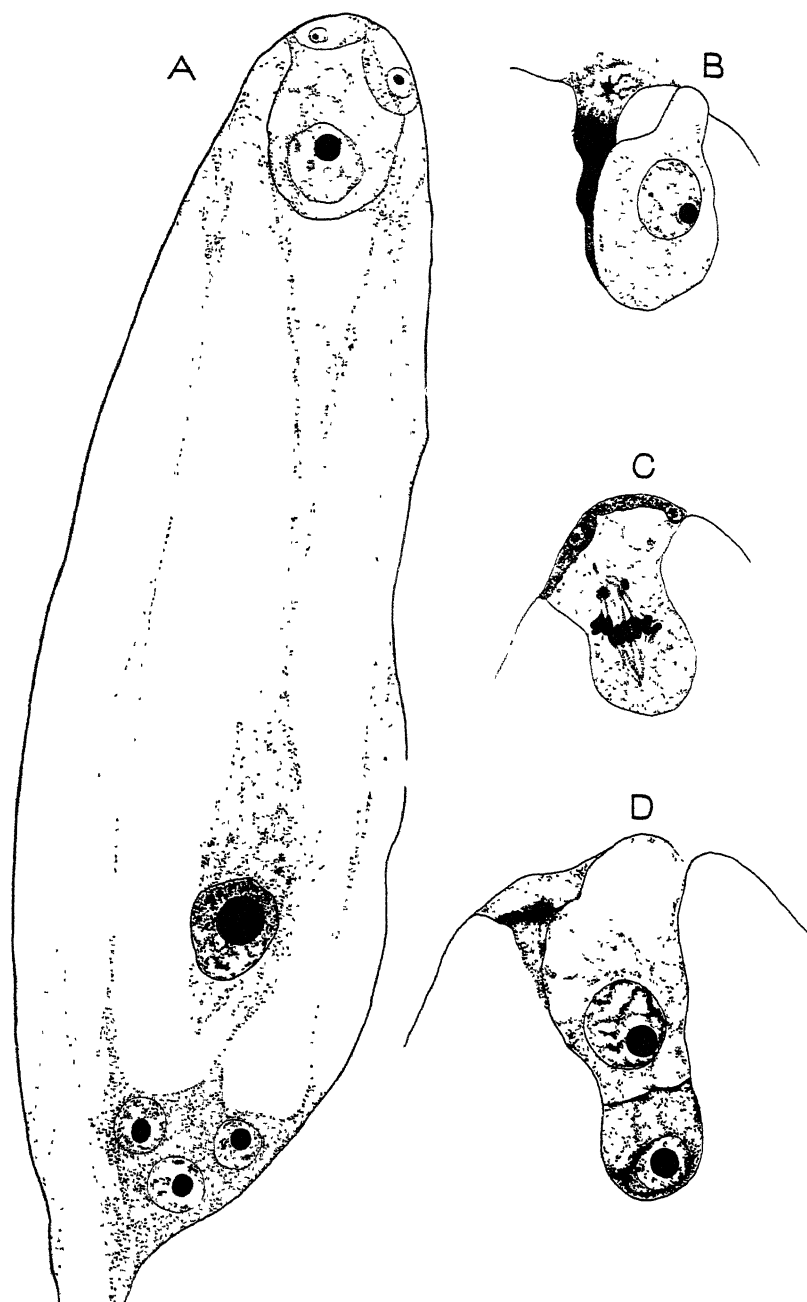
FIG. 12.—A, embryo sac at time of fertilization; B, fertilized egg with pollen tube; C, first division of the fertilized egg, D, 2-celled embryo. All × 923
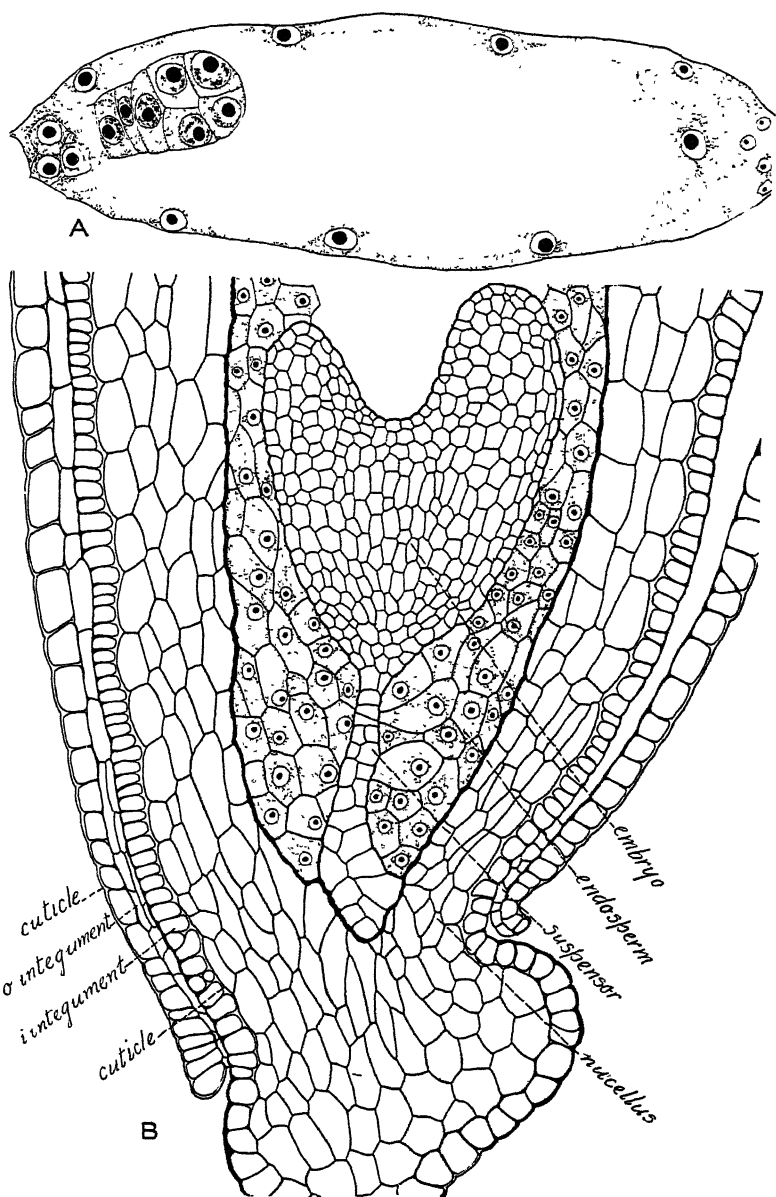
FIG. 13.—A, embryo sac with young embryo and endosperm nuclei, × 470, B, young embryo surrounded by endosperm tissue, × 225

After the embryo sac has reached its final form the peripheral layers of the nucellar tissue are from 4 to 6 cells thick. The embryo and with it the embryo sac enlarges at the expense of the inner peripheral layers which are gradually absorbed. The perisperm at this time contains only a small quantity of starch; soon, however, the cells become packed with large starch grains except in the region of the chalaza, which is always poor in reserve materials.
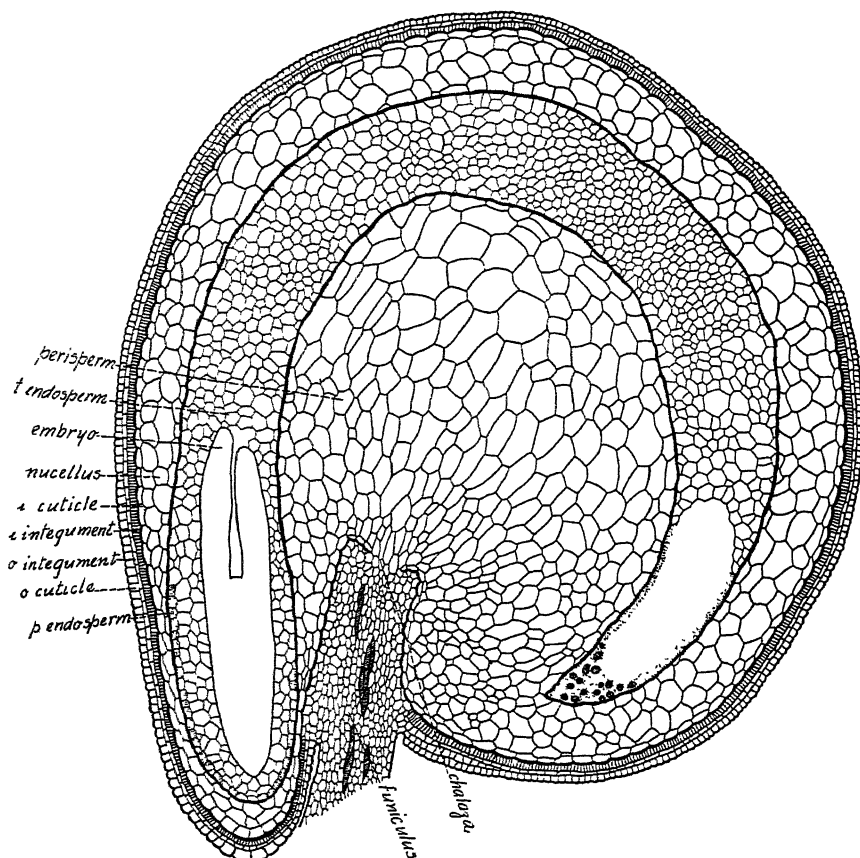
FIG. 14.—Section through ovule with young embryo. × 10

STRUCTURE OF THE SEED AND SEED COAT

The mature seed is a shiny, lentillike structure, about 3 millimeters long and 1.5 millimeters thick (fig. 15, B, and pl. 1, A). The embryo occupies a horizontal position and is curved in such a manner that it completely incloses the perisperm (pl. 1, B). The latter is formed by large isodiametric cells, which in the region of the funiculus appear more or less elongated. The lower part of the radicle is inclosed by a single-layered endosperm.

The mature embryo is protected by a seed coat which has its origin in the integuments of the ovule. The two integuments have two layers each, but the mature organ has only three layers, since the
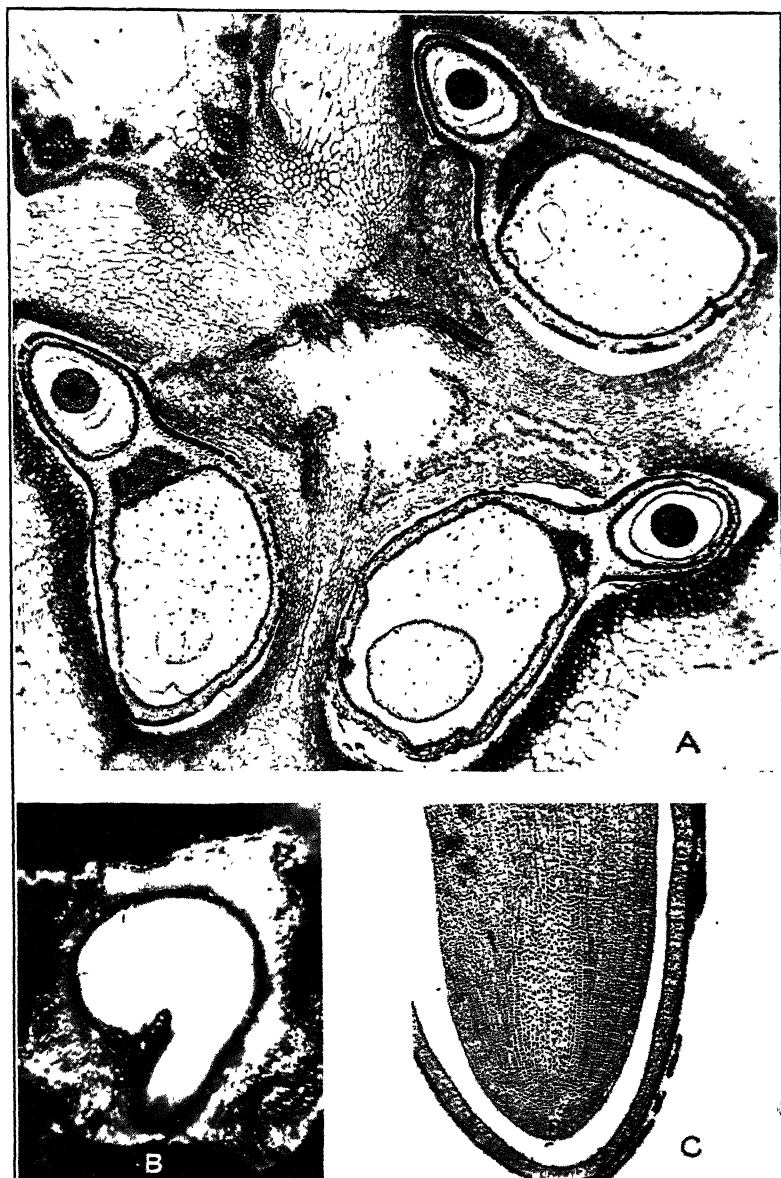
Fig. 15.—A, section through young seed ball showing three ovules with their young embryos in cross section, × 62; B, section through a mature seed, × 13; C, longitudinal section through basal part of embryo. Note the single-layered endosperm surrounding the radicle. × 77

outer layer of the inner integument degenerates during the growth of the seed. The outer integument consists at first of two undifferentiated layers, but the cells of the outer layer early enlarge while their nuclei degenerate. The outer wall begins to thicken and becomes covered by a cuticle which later becomes quite extensive. The second layer consists of small cells with large central nuclei and dense content; the individual cells are separated by small intercellular spaces. The inner integument shows in its meristematic condition nothing that would distinguish it from the outer one, but in its subsequent development it undergoes important changes. The cells of the outer layer remain small and gradually lose their content; the nuclei are obliterated and the layer becomes extinct. The cells of the inner layer enlarge and become delicately sculptured. Between inner integument and nucellus is a thick cuticle which is seen as a wavy line of highly refractive appearance. The cells of the outer integument contain starch and there is an extensive tannin deposit in both integumentary layers (fig. 15, A).

The mature outer seed coat is very brittle and separates easily from the seed. Its reddish brown color is due to the pigmentation of the outer layer. The latter is composed of polygonal, somewhat elongated cells devoid of intercellular spaces. The cells of the inner layer are smaller and when more than one layer thick they appear narrow and elongated. In the mature seed these cells are empty and more or less crushed. The mature inner testa is formed by only a single layer of cells characterized by the above-mentioned striated wall thickenings.

SUMMARY

The microsporangia begin development before the macrosporangia and precede the latter stage for stage. When the pollen is shed the embryo sac is also fully developed, but fertilization does not take place until the stigma lobes have fully expanded, which normally occurs the day after the flowers have opened. For that reason the individual flower of a cluster ordinarily is not selfed.

The nuclear phenomena offer nothing of the unusual compared to that in other plants. The appearance of the chromosomes in diakinesis is regular and there are no unpaired univalents as far as could be determined. The nuclear plate in the heterotypic anaphase contains 9 chromosomes. This number is typical for *Beta maritima* and for most of the sugar-beet material which was available for study. The anaphasic separation is also quite normal, although occasionally there is a lagging of chromosomes. There is a short interkinesis followed by a normal development of the microspores.

The megasporocyte gives rise to four megaspores of which the innermost develops into the embryo sac. The latter possesses the typical number of nuclei. About the time of fertilization the antipodals and synergids degenerate.

The embryo is curved and incloses a perisperm while the radicle is surrounded by a single-layered endosperm, the remains of a tissue which after its formation was absorbed by the developing embryo.

The seed coat consists of an outer and an inner testa derived from the two integuments of the ovule.

The median trace of the floral axis is derived from the left cauline bundle a little distance below the node and it ascends for five internodes before passing out into a leaf. The two lateral traces are also derived from their adjacent cauline bundles; they ascend for two and three internodes, respectively, before entering a leaf. This mode of origin differs from that described by Fron for *Beta cicla*.

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SOME VARIATIONS OF THE HEAT METHOD FOR STERILIZING MILKING MACHINES[1]

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INTRODUCTION

The method of sterilizing milking machines by the use of heat has been advocated only during the last few years. The chief criticism of this method has been that it is too injurious to the rubber parts of the machines.

REVIEW OF LITERATURE

Bulletin 492[2] of the New York Agricultural Experiment Station reports experiments carried on by Ruehle in 1917 on the use of hot water for the sterilization of milking machines. In these experiments the teat cups and tubes were washed by drawing cold water, hot alkali water, and clean hot water through them. They were then placed on a stove in a 30-quart covered pail containing water which was heated to 180° to 200° F. The disks and mouthpieces became useless after two days of this treatment. These were replaced, and the temperature was reduced to 160° to 170° F. At this temperature the mouthpieces and disks lasted 17 days, during which time the bacterial counts were very low. At a temperature between 150° and 160° a new set of mouthpieces and disks lasted 25 days, and another set 13 days. Bacterial counts, although still low, were higher than those in the previous tests. Ruehle reached the conclusion that the heat-sterilization method gave very good results bacteriologically but was very destructive to certain rubber parts.

In 1921, Robertson[2] repeated the tests made by Reuhle in 1917, using a temperature of 160° to 170° F. The results were about the same as those obtained by Ruehle. No mention is made of how long the rubber mouthpieces and disks lasted under chemical methods of sterilization. The bulletin mentioned reports that data obtained from control laboratories showed that at least one farm furnishing certified milk in New York State used the heat-sterilization method without undue destruction of the rubber parts.

Reports from laboratory tests conducted in 1918 and 1921 on rubber parts obtained from seven generally used milkers showed that the rubber parts supplied with the majority of milkers were more resistant to heat than was commonly believed. This applied particularly to the best grade of cloth-wrapped, pole-lined, steam-vulcanized tubing, but did not apply so well to the tubing vulcanized in iron molds. It was also noted that in some instances with some metals the rubber apparently melted and stuck to the metal parts.

These tests were made under laboratory conditions only, and the parts were subjected to each of the following conditions daily for 20 days: Under steam pressure in autoclave, in flowing steam, in boiling water, and in hot water at 160° to 170° F. It was not stated whether one method was more or less detrimental than another. The final conclusion was that heat sterilization could be recommended as an effective method for those machines which were, or could be, equipped with rubber parts that would withstand heating twice a day for a reasonable length of time.

In 1920 Hart and Stabler [3] published a report of their experiments in California with the heat-sterilization method. Tests were conducted at two high-class dairies and one ordinary dairy at a temperature of 160° to 190° F. for 15 to 30 minutes. Excellent results were obtained at the two high-class dairies, but the bacterial counts at the ordinary dairy were much higher, running in to the hundreds of thousands. They were lower in number, however, than those obtained before the heat-sterilization method was used. From these tests Hart and Stabler came to the conclusion that "heat sterilization is the only way to sterilize successfully milking machine rubber parts under ordinary ranch conditions." They reported the length of life of the rubber parts at one high-class dairy to be from two to four months, the tubing lasting longer than the inflations.

In 1923, the writer [4] published the results of tests on the heat method of sterilization at temperatures of 160° to 170° F. The parts, after being washed, were submerged in water at these temperatures and allowed to remain undisturbed until the next milking, the water cooling gradually. The results obtained at 20 farms showed the effectiveness of this method of sterilization. The length of life of teat-cup liners ranged from 6 to 17 weeks.

In 1925 the results were published of investigations made by the writer [5] on the comparative value of the heat method, the chlorine method, and the salt and chlorine method of sterilizing milking machines. These tests showed that the heat method, under conditions as nearly identical as possible, gave more uniform and appreciably lower bacterial counts than did the other two methods.

PRESENT INVESTIGATIONS

EXPERIMENTAL METHODS

In order to find a method that would give as good results bacteriologically as the heat method but with an increase in the length of life of the rubber parts, the present investigations were undertaken.

In the heat method referred to, the unit, consisting of teat cups, claw, and tubing, after being washed is placed in hot water at a temperature of 160° to 165° F., and allowed to remain there between milkings, the water cooling gradually.

Three single units were used in these tests. They were washed, sterilized,[6] and handled personally by the writer during the entire

[3] HART, G. H., and STABLER, W. H. EXPERIMENTS WITH AND PRACTICAL APPLICATION OF HEAT STERILIZATION FOR ALL PARTS OF MILKING MACHINES. Jour. Dairy Sci. 3: 33–51. 1920.
[4] BURGWALD, L. H. CLEANING MILKING MACHINES. U. S Dept. Agr. Farmers' Bul 1315, 16 p., illus. 1923
[5] BURGWALD, L. H CLEANING MILKING MACHINES Jour Agr Research 31· 191–195. 1925
[6] The term "sterilize" used in this paper indicates a condition of practical rather than of absolute sterilization.

period of each test, each unit receiving exactly the same treatment except for the method of sterilizing or the disposition after sterilization.

Immediately after milking, each unit was rinsed by drawing clean, cold water through by vacuum, washed with a brush in hot water (110° to 120° F.) containing washing powder, and rinsed in clean, hot water. The buckets and heads were rinsed in cold water, washed with a brush in hot water containing washing powder, and rinsed in hot water at a temperature above 160° F. They were again rinsed with hot water (above 160° F.) before using.

The units were taken apart after every sixth to eighth milking, and the rubber parts, consisting of teat cups, claw, and tubing were thoroughly washed. In a few cases they were taken apart only once a week.

EFFECT OF DIFFERENT METHODS OF HANDLING MILKING UNITS ON THE BACTERIAL COUNT OF THE MILK

Throughout one set of tests the units were sterilized as follows:

All the units were placed in a tank of hot water at a temperature ranging from 160° to 167° F., the average temperature being about 163°, and the water was allowed to cool gradually. At the end of 20 to 35 minutes, the average time being 30 minutes, two of the units, Nos. 1 and 2, were removed. No. 1 was placed in a refrigerator, and No. 2 was placed in a weak solution of chlorinated lime. A stock solution was made by dissolving a 12-ounce can of chlorinated lime, containing 24 per cent available chlorine, in 1 gallon of water. This was filtered into a glass bottle, stoppered, and kept in a dark, cool place. The soak solution (about 1:20,000), in which unit No. 2 was kept between milkings, was made by using 1 ounce of the stock solution to 3 gallons of water and was made fresh daily. Unit No. 3 remained in the hot water between milkings.

In winter the temperature of the refrigerator in which unit No. 1 was placed after sterilization ranged from 32° to 48° F., the average being 35°. Temperature readings were taken both when the unit was put in and when it was taken out. The temperature of the weak chlorine solution in which No. 2 was kept between milkings and of the water in which No. 3 was kept ranged from 32° to 62°, the average being 45° at the time the units were removed. The bacterial counts of milk drawn in winter with machines handled as described above are given in Table 1.

TABLE 1.—*Summary of bacterial counts obtained in winter from samples of milk drawn with machines handled in various ways after sterilizing*

Method of handling after sterilizing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No. 1, placed in refrigerator_____	188	300 to 10,900	3,130	187	99.5
Unit No. 2, placed in weak chlorine solution_____	186	200 to 9,300	2,320	186	100.0
Unit No. 3, remaining in hot water_____	184	300 to 7,400	2,660	184	100.0

In summer the temperature of the refrigerator in which unit No. 1 was placed after sterilization ranged from 34° to 60° F., the average

temperature being 40°. Temperature readings were taken both when the unit was put in and when it was taken out. The majority of readings were 36° to 44°. The temperature of the weak chlorine solution in which No. 2 was kept and of the water in which No. 3 was kept between milkings ranged from 60° to 86°, the average temperature being 73° at the time the units were removed. The bacterial counts of milk drawn in summer with machines handled as here described are given in Table 2.

TABLE 2.—*Summary of bacterial counts obtained in summer from samples of milk drawn with machines handled in various ways after sterilizing*

Method of handling after sterilizing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count ot 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No 1, placed in refrigerator	108	600 to 10, 700	3, 080	107	99 1
Unit No 2, placed in weak chlorine solution	106	300 to 5, 800	1, 990	106	100. 0
Unit No. 3, remaining in hot water	106	300 to 5, 700	2, 410	106	100 0

The results of the winter and summer tests combined are shown in Table 3.

TABLE 3.—*Summary of bacterial counts (Tables 1 and 2 combined)*

Method of handling after sterilizing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No 1, placed in refrigerator	296	300 to 10, 900	3, 110	294	99 3
Unit No. 2, placed in weak chlorine solution	292	200 to 9, 300	2, 200	292	100. 0
Unit No 3, remaining in hot water	290	300 to 7, 400	2, 570	290	100 0

There was little difference in the results obtained by the three methods as shown in Tables 1, 2, and 3. All were good. The unit placed in a weak chlorine solution after sterilization gave somewhat lower counts than were secured by the other methods, while the unit placed in the refrigerator gave somewhat higher counts. However, the differences were not significant.

In another set of tests with three units, one unit (No. 4) was sterilized in hot water at a temperature of 145° to 150° F., the average temperature being 148°, and allowed to remain therein between milkings, the water cooling gradually. The other two units (Nos. 3 and 5) were sterilized in hot water at a temperature of 160° to 165°, the average temperature being 162°. At the end of 20 to 45 minutes (average 33 minutes) unit No. 5 was removed, hung in a warm room, and protected from dirt and contamination. Unit No. 3 remained in the hot water between milkings, the water cooling gradually, and was used as a control. The temperature of the room in which unit No. 5 was hung after being removed from the hot water ranged from 70° to 82° F., the average temperature being 75.5° F. The results of these experiments are shown in Tables 4 and 5.

TABLE 4.—*Summary of bacterial counts of samples of milk drawn with units sterilized at different temperatures*

Sterilization temperature	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No 4, 145° to 150°F	142	3,000 to 51,000	11,930	73	51
Unit No. 3 (control), 160° to 165°F	40	700 to 6,000	2,520	40	100

TABLE 5.—*Summary of bacterial counts of samples of milk drawn with units sterilized at 160° to 165° F., and receiving different subsequent treatment*

Disposition of unit after sterilizing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No. 5, left in hot water for 20 to 45 minutes, then hung in warm room	154	1,100 to 24,000	5,540	137	89
Unit No. 3 (control), remaining in hot water	40	700 to 6,000	2,520	40	100

The unit sterilized at 145° to 150° F. gave somewhat higher counts than were obtained by the other methods. The unit heated to 160° to 165° for 20 to 45 minutes and then hung in a warm room gave good results, although somewhat higher counts than those shown in Tables 1, 2, and 3.

As a rule, the units were taken apart and thoroughly washed twice a week, but on a few occasions they were taken apart only once a week. Whether they were taken apart once or twice a week, however, seemed to make little if any difference in the bacterial count of the milk, as is shown in Table 6.

TABLE 6.—*Effect of taking units apart frequently for washing, as shown by bacterial counts of milk drawn with a unit sterilized at 160° to 165° F. and allowed to remain in the water between milkings*

Day No	Number of milkings since unit was taken apart for washing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Average bacterial count per cubic centimeter for total number of samples on first three and on last three days
1	1	39	400 to 7,400	2,910	
	2	41	700 to 5,400	2,660	
2	3	40	400 to 6,500	2,410	
	4	40	300 to 6,700	2,510	2,580
3	5	36	300 to 6,300	2,410	
	6	36	400 to 6,500	2,570	
4	7	28	700 to 4,700	2,200	
	8	25	1,100 to 5,000	2,540	
5	9	14	900 to 5,900	2,660	2,520
	10	7	1,400 to 5,000	2,590	
6	11	11	1,400 to 5,000	2,860	
	12	12	800 to 6,000	2,740	
	Total	329	300 to 7,400	2,560	

It was noted, however, that the rubber tubing had a tendency to stick to the metal parts at times when the unit was taken apart only once a week.

EFFECT OF DIFFERENT METHODS OF HANDLING MILKING UNITS ON THE LENGTH OF LIFE OF THE RUBBER PARTS

A record was kept of the length of life of the rubber parts when different methods of sterilization were employed. The machine used was of the molded rubber teat-cup liner type. The units were generally used to milk four or five cows each at a milking for two milkings a day. Occasionally, however, they were used for only one milking a day.

The teat-cup liners were the first rubber parts to wear out, the rubber tubing outlasting them considerably. The results of tests to determine the length of life of teat-cup liners under different methods of treatment are given in Table 7.

TABLE 7.—*Length of life of teat-cup liners under various methods of sterilization*

	Length of life of teat-cup liners	
Method of sterilizing	Number of milkings	Number of days allowing two milkings a day
Heat method, 160° to 167° F , remaining in water between milkings	175	87.5
Do	188	94.0
Heat method, 160° to 167° F , 20 to 35 minutes, and placed in refrigerator	315	157 5
Heat method, 145° to 150° F , remaining in water between milkings	218	109. 0

As here shown the average life of the teat-cup liners was about three months when sterilized at 160° to 167° F. and allowed to remain in the water between milkings. Removing the unit at the end of 20 to 35 minutes and placing it in a refrigerator increased the length of life of the teat-cup liners to a little over five months. The bacterial counts were almost equally as low as those obtained when the unit remained in hot water between milkings, as shown in Tables 1, 2, and 3.

When a sterilizing temperature of 145° to 150° F. was used and the unit was allowed to remain in the water between milkings, the water cooling gradually, the length of life of the teat-cup liners was increased to 109 days. Bacterial counts of the milk samples drawn with this unit were not so low, however, as those obtained by the other methods. Nearly 50 per cent of the samples had a count of over 10,000 per cubic centimeter, as shown in Table 4.

The short rubber milk tubes on the unit heated to 160° to 167° F. and remaining in the water between milkings, lasted for 332 milkings, or 166 days; while the short air tubes and long milk tube lasted for 432 milkings, or 216 days. The tubing on the machines sterilized by the other methods was still in good condition at the end of 267 days, when the tests were discontinued.

A unit sterilized in a saturated brine and chlorine solution (1 : 5,000) was also used. The teat-cup liners of this unit were employed for 534 milkings, or 267 days, and were still in excellent condition and probably good for a few more months when the tests were discontinued.

SUMMARY

Heating a unit in water at a temperature of 160° to 167° F. for 20 to 35 minutes and then removing it to a refrigerator and keeping it there between milkings increased the length of life of the rubber parts materially over those left in the hot water between milkings. Bacterial counts on samples of milk drawn with the unit which was placed in the refrigerator were very low, being only slightly higher than those in milk drawn with the unit which was left in the hot water.

Bacterial counts made on samples of milk drawn with a unit sterilized at the same temperature and for the same length of time as the one placed in the refrigerator, but placed in a weak chlorine solution (about 1:20,000) between milkings, were even lower than those obtained with the unit which remained in hot water.

Bacterial counts made on samples of milk drawn with the unit sterilized at a temperature of 160° to 165° F. for 20 to 45 minutes, removed and hung in the warm washroom, and protected from dust and contamination between milkings, were fairly low, although considerably higher than those obtained on milk drawn with the unit which remained in hot water between milkings.

The experiments indicate that sterilizing the units at a temperature of 160° to 167° F. for 20 to 35 minutes and placing them in a weak chlorine solution (about 1:20,000) or hanging them in a refrigerator or cold place (below 50°) protected from dust or contamination, will give excellent bacterial results and that the life of the rubber parts will be materially longer than when the units are allowed to remain in the hot water between milkings. Using the same treatment but hanging the units in a clean, warm place gave low bacterial counts, although not quite so low as those obtained by the other methods.

At a temperature of 145° to 150° F. the life of the rubber parts was somewhat longer than at a temperature of 160° to 165°, but the bacterial counts were not nearly so low as at the higher temperatures.

The rubber tubing always outlasted the teat-cup liners.

The heat method of sterilization or any of its variations shortened the life of the rubber parts more than the salt and chlorine methods.

From a bacteriological standpoint, taking the units apart twice a week seemed to have no advantage over once a week when the heat method was used for sterilizing. The rubber tubing, however, had a tendency occasionally to stick to the metal parts when the units were taken apart only once a week.

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A BIOCHEMICAL STUDY OF THE FALSE-BLOSSOM DISEASE OF THE CRANBERRY [1]

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INTRODUCTION

Although the false-blossom disease of cranberries has been recognized and studied for at least 20 years, its cause remains undetermined. Numerous theories have been advanced to explain its nature, and various control measures have been tried without much success. Lack of drainage, drought, and excessive nitrogen content of the bogs have been blamed for the trouble, but none of these conditions is sufficiently well correlated with the presence of the disease to be regarded as the causal agent. It was thought that a chemical study of diseased and healthy plants might reveal some marked differences in metabolism and perhaps throw more light on whether the disease is due to faulty nutrition. It was hoped further that such a study might lead to a point of attack by which the cause of the disease might be determined and methods for its control or prevention worked out.

DESCRIPTION OF DISEASED PLANT

The disease has been described best by Shear (*10*) [3] in a paper which contains detailed plates and cuts of all stages of the disease. In part, he describes the pathological condition as follows:

"The disease under consideration produces as one of its most conspicuous features a malformation or metamorphy of the floral organs. * * * In the simplest form of the trouble the flower pedicels become more or less erect instead of drooping and the calyx lobes become enlarged, greenish, and somewhat foliaceous. The petals become shortened, broadened, and slightly reddish or greenish in color. * * * The stamens and pistil are more or less aborted and malformed and no fruit is produced.

"All intermediate gradations of phyllody can usually be found among diseased vines, from the simple form, in which there is only a shortening and thickening of the parts of the perianth, to cases in which the entire flower is replaced by a short branch with small leaves.

"Besides the transformation of the floral organs, other abnormalities of growth are usually found. Affected plants have a great tendency to develop lateral branches from the usually latent axillary buds situated on the vine below the fruit bud. * * * The branches are slender and weak and fail to produce normal flowers or fruit. They give the plant a kind of witches'-broom appearance. In some

instances the end of the flowering shoot, instead of forming a fruit bud for the next season, as is the case in normal plants, continues to grow and produces a long, slender runner. Cranberry plants in bogs where this malformation occurs generally show an excessive vegetative growth, usually forming a deep, dense mass of vines. In their dormant condition the terminal buds are frequently enlarged and abnormal and die during the winter."

HISTORY AND ECONOMIC IMPORTANCE OF THE DISEASE

The first published records of false-blossom disease appeared in 1908 in a report by C. L. Shear (*9*) read before the Wisconsin Cranberry Growers Association. The disease had been common in Wisconsin for some time previous to that date. It seems to have originated there and to have spread to the other cranberry-growing sections in plantings of Wisconsin nursery stock. In Wisconsin false blossom causes serious loss in production each year. In Massachusetts it is rapidly becoming serious, and in New Jersey also it has gained some headway. An idea of the importance of the disease may be had from a summary of the nursery inspections made in Wisconsin during the years 1915 to 1919. Of 252 acres, the average acreage inspected annually, 54.2 acres, or 21.5 per cent, was found to be infected.

The first report of the disease in the Cape Cod section of Massachusetts was made in 1914, when infected vines were found on five bogs. Although all these plants were destroyed, six new infestations were reported in 1919. In practically all cases where the history of the plantings could be traced it was discovered that the vines originally came from Wisconsin. In 1923, Franklin, of the Massachusetts Cranberry Experiment Station, had a list of 25 infected bogs within a radius of 20 miles of the State bog at Wareham, Mass.

NATURE AND CAUSE OF THE DISEASE

From the very beginning the false-blossom disease was thought to be caused by poor cultural conditions. Shear (*9*) in 1908 pointed out that there appeared to be no fungus connected with the disease, but that "it seems probable that it is due to an unusual disturbance of the physiological functions of the plants, brought about by abnormal conditions of growth and an excessive amount of nitrogenous plant food, derived chiefly from the great quantity of decaying vegetable matter present and associated with an excess of water. These may stimulate a highly abnormal vegetative growth throughout the season and so unbalance the plant that it is unable to produce normal flowers and fruit but instead attempts to change them into vegetative organs." He recommended pruning of the vines, liberal sanding, and better drainage.

Later, in 1914, after making a critical survey of the situation in Wisconsin, Jones and Shear (*5*) came to similar conclusions. At that time they reported that "no evidence exists that the malady is due in any way, directly or indirectly, to the attacks of a parasite, insect, or fungus." They also pointed out that in Wisconsin it does not occur where the best cultural practices are carried out and that the disease disappears when the diseased plants are grown under better cultural conditions. They conclude that apparently the most

important factor in the elimination of false blossom is proper drainage. Shear (*10*) has also observed that where the disease is prevalent there is a deep, coarse peat soil, supplied with excessive water during the greater part of the season. However, Malde (*7*) has noted that the development of the disease is also favored by conditions of extreme drought.

Thus, it appears that the actual cause of the phyllody is still a question to be answered. Certain cultural conditions seem to favor its development, but not consistently enough to be taken as a positive source of the trouble. The transposition of the disease from Wisconsin to other sections of the country leads one to suspect that some organism may be the cause, but thus far no such agent has been found.

As great uncertainty prevails concerning control measures for the disease as concerning the source of the trouble, proper drainage, sanding the bogs, pruning the vines, and, in the most serious cases, scalping and burning over the bogs have been recommended (*10*). Malde (*7*) believes an application of rock phosphate to be beneficial in Wisconsin. However, no one of these remedies, or even all of them, give positive assurance of healthy vines free from false blossom in the future.

In some cases diseased plants that were transplanted and kept under better cultural conditions regained their healthy and fruitful condition. But this recovery was not consistent. It occurred in some cases, while in others the plants remained unfruitful. The disease has been observed on some of the best kept bogs in Massachusetts, in bogs on which the best cultural methods are practiced. It has developed on bogs which have a high moisture content and also on those which have a low moisture content.

MATERIALS AND METHODS OF ANALYSIS

Since the samples were collected in three different years, and since changes and minor improvements were made each year in the methods of sampling, it seems advisable to describe each collection of samples separately.

The samples collected in 1920 were taken by Neil E. Stevens, of the Bureau of Plant Industry, United States Department of Agriculture. Samples of diseased plants were selected from the Vaughn Bog in North Carver, Mass., and those of healthy plants from the Massachusetts State Experiment Station Bog in East Wareham, Mass. All the samples were taken between 10 a. m. and 2 p. m. on clear days. The diseased samples were taken July 14 and 15; the healthy samples July 9 and 11. After collection, the leaves were at once separated from the stems and the weighed samples were placed in hot 95 per cent alcohol containing half a gram of precipitated calcium carbonate to neutralize acidity and were boiled for one hour. The samples were then transported to the New Hampshire Experiment Station by auto and left in the alcohol in quart jars until analyzed.

Samples were again taken in 1922 from the same bogs. That year the samples were all taken the same day, June 28. The diseased plants were sampled between 10 a. m. and noon, and the healthy plants were sampled between 1 and 2 p. m. The samples were brought to the Massachusetts State Cranberry Experiment Station,

where the leaves were at once separated from the stems (the blossoms were included with the leaves), and samples of each were weighed, placed in boiling 95 per cent alcohol containing precipitated calcium carbonate, and boiled for one hour. The samples were taken by auto to the New Hampshire Experiment Station to be analyzed.

In 1923 still more extensive samples were taken. Three bogs infected with the disease were selected, namely, the Cornfield and Thyonnett Bogs, both in East Wareham, Mass., and the Vaughn Bog, in North Carver, Mass. Samples of healthy and diseased plants were taken between 7 and 9 a. m., July 7. The samples were at once brought to the laboratory and the leaves and stems were separated. After the samples had been weighed out they were placed in hot 95 per cent alcohol containing 0.5 gram of precipitated calcium carbonate and boiled for one hour. The stems and leaves were all separated, and by 3 p. m. the samples were preserved in alcohol. The sampling was carried out by members of the Massachusetts Cranberry Experiment Station staff and the New Hampshire Experiment Station staff. The samples were transported by auto to the New Hampshire Experiment Station to be analyzed.

The method of putting up samples described above seems to be the least objectionable for preserving material on which carbohydrate determinations are to be made. In 1920 and 1922 additional samples for tissue analysis were put up. These samples received the same treatment as those already described, except that no calcium carbonate was added.

In determining the carbohydrates, the samples preserved in alcohol containing calcium carbonate were used. These samples, in most cases, consisted of 150 grams of leaves and 75 grams of stems.

The alcohol in which the plant tissue had been preserved was poured off the sample through a filter into a liter volumetric flask. The stems, after being dried in a vacuum oven overnight, were cut with pruning shears into pieces about a centimeter in length. All samples were then removed to 500 c. c. beakers, and just enough 80 per cent alcohol was added to cover them. The beakers were placed on the steam bath, and the liquid was brought to a boil for a few minutes. The beakers were then removed and allowed to come to room temperature, after which the extract was poured through the filter into the liter volumetric flask. This extraction was repeated until the volume of extract was approximately 1,900 c. c. The residue was dried in a vacuum oven at 65° C. and then removed from the beakers for grinding. The empty beakers were cleaned with alcohol, and the filter paper was thoroughly washed into the volumetric flask. The extract was brought to volume at 20° and removed to 2-liter glass-stopped bottles, which were carefully sealed with paraffin.

The residue from the extraction, after being dried in the vacuum oven, was allowed to come to an air-dry condition in the laboratory in dry, weighed beakers. Just before the samples were ground, they were weighed accurately. They were ground very fine in a coffee mill and placed immediately in glass-stoppered bottles. Aliquots of one-fifteenth of the air-dry weights were then weighed into small glass vials for analysis.

In determining the sugars, one-fifteenth aliquots of the residue and extract were taken for analysis. The extract was carefully evap-

orated almost to dryness at a low temperature, to avoid destroying the sugars; it was taken up with a little water; and all the alcohol was driven off. The residue was placed in a beaker, and warm water having a temperature of about 40° C. was added and the dry material thus soaked. This was then placed on a filter and extracted with successive small portions of warm, distilled water, the water extract being added to the beaker containing the evaporated alcohol extract. When the total volume was about 200 c. c. the extract was transferred to a 250 c. c. volumetric flask. The residue on the filter paper was saved for the starch and acid hydrolyzable determinations. The extract was cleared with a small amount of lead acetate, between one-half of 1 c. c. and 1 c. c. being used. After being brought to 20°, the extract was made to volume and the lead precipitate filtered off. The filtrate was then deleaded by the addition of just enough sodium oxalate to precipitate the excess of lead present. This precipitate was filtered off, the solution was brought to 20°, and the reducing sugars were determined.

The reducing power of all of the sugar solutions was determined by a modification of the Bertrand and Munson and Walker methods (*8*). A 25 c. c. aliquot of the cleared solution was used to determine the reducing substances present. A 50 c. c. aliquot of the cleared solution was hydrolyzed by the citric-acid method of Davis and Daish (*2*) for the determination of sucrose. The difference in reduction before and after hydrolysis was calculated and expressed as percentage of dextrose.

The residue left after the water extraction of the samples in the determination of free reducing substances was used for the determination of starch and acid hydrolyzable material. This residue was washed into a 250 c. c. wide-mouthed Erlenmeyer flask with 190 c. c. of distilled water and placed on a boiling water bath for half an hour to gelatinize the starch. The flask was then cooled to 38° C., and 10 c. c. of a 1 per cent solution of takadiastase was added to the solution and mixed thoroughly. A little toluol was then added, and the flask was stoppered and placed in a water bath in which the temperature was accurately controlled at 38°. At the end of 24 hours the flask was removed to a boiling water bath for about 20 minutes to inactivate the enzyme. The solution was then filtered into a 500 c. c. volumetric flask and the residue washed with successive portions of hot distilled water until a volume of 450 c. c. was obtained. The residue was returned to the Erlenmeyer flask and run with new enzyme for another 24 hours to make sure that all the starch had been hydrolyzed. The residue after the second hydrolysis was reserved for the acid-hydrolyzable determination. The filtrate from each hydrolysis was cooled to 20°; a small quantity of neutral lead acetate was added; the filtrate was made up to mark and filtered. To the solution was then added sodium oxalate to precipitate the excess lead, and the lead oxalate was filtered off. Fifty c. c. portions of the filtrate were used in estimating the reducing power due to hydrolyzed starch and the results were calculated and stated as dextrose. A blank experiment for each enzyme was carried through for each set of determinations.

In the determination of acid-hydrolyzable material, the residue from the starch determination was transferred to a 600 c. c. Erlenmeyer flask, to which 142 c. c. of distilled water and 8 c. c. of concentrated hydrochloric acid were added. The flask was then boiled

gently for $2\frac{1}{2}$ hours under a reflux condenser. At the end of that time the solution was filtered into a 500 c. c. volumetric flask and washed with hot water until a volume of 450 c. c. was obtained. This solution was then cooled and neutralized to litmus with sodium hydroxide, and just enough lead acetate was added to clear the solution. The solution was made up to mark at 20° C. It was then filtered, the excess lead was precipitated with sodium oxalate, and the lead oxalate was filtered off. A 25 c. c. aliquot of this solution was used to estimate the reducing power due to acid-hydrolyzable material, and the results were stated as dextrose.

The Kjeldahl method (*1*), modified to include nitrates, was used in determining the total nitrogen.

The phosphorus determinations were made by the Neumann-Pemberton method (*8*).

The method in tissue analysis was based mainly on the procedures employed by Koch (*6*) in his analysis of brain material. The method as outlined is a modification of the original Koch method. It is essentially the separation of the alcohol-preserved material into three subfractions, viz, fraction F_1, or lipoids, fraction F_2, or water soluble, and fraction F_3, or alcohol and water insoluble. Each fraction thus separated was analyzed chemically for the various constituents of interest.

RESULTS

All the analyses presented are the averages of two or three determinations (in most cases made on separate samples) which checked within 5 per cent or less of the total amount of the constituent found.

Table 1 gives the percentage of free reducing substances in the leaves and stems of the diseased and healthy plants. In every case the amount of reducing sugars is higher in the diseased than in the healthy leaves, the difference ranging from 20 to 60 per cent. Similarly, there was a larger amount of reducing sugars in the stems of the diseased plants than in the stems of the healthy plants (except those tested in 1922), the differences ranging from 6 to 37 per cent.

TABLE 1.—*Free reducing substances in tissues of healthy and diseased cranberry plants, expressed as percentages of dextrose*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Percentage of free reducing substances			
			In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog [a]	Leaves	2.76	2.30	+0.46	+20.00
June, 1922	do [a]	do	2.85	2.18	+.67	+30.73
July, 1923	Cornfield Bog	do	2.85	2.17	+.68	+31.33
Do	Vaughn Bog	do	3.24	2.39	+.85	+35.56
Do	Thyonnett Bog	do	3.28	2.04	+1.24	+60.78
July, 1920	Vaughn Bog and State bog [a]	Stems	1.33	1.25	+.08	+6.40
June, 1922	do [a]	do	.50	.65	−.15	−23.07
July, 1923	Cornfield Bog	do	1.07	.83	+.24	+28.91
Do	Vaughn Bog	do	1.17	.98	+.19	+19.38
Do	Thyonnett Bog	do	1.14	.83	+.31	+37.34

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The values for sucrose are presented in Table 2. In 1920 there was no difference in the sucrose content of the diseased and healthy leaves, and in 1922 the healthy leaves were higher in sucrose. In 1923, however, on each of the three bogs sampled the diseased leaves were considerably higher in sucrose, the differences ranging from 36 to 113 per cent. In all instances it was found that the diseased stems were higher in sucrose than were the healthy stems, the differences ranging from 28 to 147 per cent.

TABLE 2.—*Sucrose content of tissues of healthy and diseased cranberry plants, expressed as percentage of dextrose*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Sucrose content			
			In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog a	Leaves	0 51	0 51	0 00	0 00
June, 1922	do.a	do	1. 34	1. 74	− 40	−22 98
July, 1923	Cornfield Bog	do	1 83	1. 16	+. 67	+57 75
Do	Vaughn Bog	do	1. 69	. 79	+. 90	+113. 92
Do	Thyonnett Bog	do	1 39	1 02	+. 37	+36. 27
July, 1920	Vaughn Bog and State bog a	Stems	. 57	. 23	−. 34	−147. 82
June, 1922	do.a	do	50	. 39	+ 11	−28 20
July, 1923	Cornfield Bog	do	1. 20	. 75	+. 45	+60. 00
Do	Vaughn Bog	do	1. 02	. 54	+. 48	+88 88
Do	Thyonnett Bog	do	1. 03	. 51	+ 52	+101. 96

a Healthy plants from the State bog; diseased plants from Vaughn Bog.

The results of the starch determinations are given in Table 3. In all the samples except those collected in 1922 the diseased leaves and stems were very much higher in starch than the healthy ones. The starch in the diseased leaves was from 99 to 559 per cent more than the quantity in the healthy leaves.

TABLE 3.—*Starch content of tissues of healthy and diseased cranberry plants, expressed as percentage of dextrose*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Starch content			
			In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog a	Leaves	2. 63	1. 32	+1. 31	+99. 24
June, 1922	do a	do	2 45	2. 89	− 44	−15. 22
July, 1923	Cornfield Bog	do	3 53	. 57	+2. 96	+519. 29
Do	Thyonnett Bog	do	2. 90	. 44	+2 46	+559. 09
Do	Vaughn Bog	do	1. 98	. 58	+1. 40	+241. 37
July, 1920	Vaughn Bog and State bog a	Stems	. 65	. 24	+. 41	+170. 83
June, 1922	do.a	do	. 10	. 80	− 70	−87. 50
Do	Cornfield Bog	do	1. 82	. 90	+. 92	+102. 22
Do	Vaughn Bog	do	. 63	. 16	+. 47	+293. 75
Do	Thyonnett Bog	do	1. 16	. 38	+. 78	+205. 26

a Healthy plants from the State bog; diseased plants from Vaughn Bog.

Table 4 shows the results of the acid-hydrolyzable determinations. In all cases the diseased leaves and stems are higher in acid-hydrolyzable substances than the healthy ones, the differences ranging from 3 to 77 per cent.

TABLE 4.—*Acid-hydrolyzable substances in tissues of healthy and diseased cranberry plants, expressed as percentage of dextrose*

[Based on fresh weight]

			Acid-hydrolyzable content			
Date of sampling	Source of material	Part of plant	In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	State bog and Vaughn Bog [a]	Leaves	4 08	3 42	+0 66	+19 29
June, 1922	do.[a]	do	3.86	3 72	+ 14	+3 76
July, 1923	Cornfield Bog	do	5.47	3 09	+2 38	+77 02
Do	Vaughn Bog	do	3 62	2. 63	+ 99	+37 64
Do	Thyonnett Bog	do	3 08	2 74	+ 34	+12 40
July, 1920	Vaughn Bog and State bog [a]	Stems	8 35	7. 41	+ 94	+12. 68
June, 1922	do.[a]	do	5 92	5 48	+ 44	+8 02
July, 1923	Cornfield Bog	do	9 84	8. 07	+1. 77	+21.93
Do	Vaughn Bog	do	8 17	6. 38	+1 79	+28 05
Do	Thyonnett Bog	do	9. 01	7. 01	+2 00	+28. 53

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog

The total carbohydrates are given in Table 5. The figures were obtained by adding the percentages of all of the previously mentioned carbohydrates. In all the samples except those taken in 1922 the diseased leaves and stems contained a larger total of carbohydrates than did the healthy leaves and stems.

TABLE 5.—*Total carbohydrate content of tissues of healthy and diseased cranberry plants, expressed as percentage of dextrose*

[Based on fresh weight]

			Carbohydrate content			
Date of sampling	Source of material	Part of plant	In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog [a]	Leaves	9 98	7 55	+2 43	+32. 19
June, 1922	do.[a]	do	10 50	10 53	− 03	− 28
July, 1923	Cornfield Bog	do	13 68	6.99	+6 69	+95. 71
Do	Vaughn Bog	do	11. 45	6 25	+5 20	+83 20
Do	Thyonnett Bog	do	9. 73	6 38	+3. 35	+52 51
July, 1920	Vaughn Bog and State bog [a]	Stems	10 90	9 13	+1 77	+19. 39
June, 1922	do [a]	do	7. 02	7. 32	−. 30	−4. 10
July, 1923	Cornfield Bog	do	13 93	10 55	+3. 38	+32 04
Do	Vaughn Bog	do	10.99	8 06	+2. 93	+36. 35
Do	Thyonnett Bog	do	12. 34	8. 73	+3 61	+41 35

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The data for the total nitrogen determinations are given in Table 6. In some instances the diseased leaves and stems were higher and in others lower in total nitrogen than the healthy ones. No consistent significant differences in total nitrogen content were found.

TABLE 6.—*Total nitrogen content of tissues of diseased and healthy cranberry plants*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Percentage of nitrogen			
			In diseased plant	In healthy plant	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog [a]	Leaves	0 400	0 484	−0. 084	−17. 35
June, 1922	do.[a]	do	.404	.383	+. 021	+5 48
July, 1923	Cornfield Bog	do	.401	.459	− 058	−12. 63
Do	Vaughn Bog	do	.421	.490	−. 069	−14. 08
Do	Thyonnett Bog	do	.401	.346	+. 055	−15. 89
July, 1920	Vaughn Bog and State bog [a]	Stems	.310	.284	+. 026	+9. 15
June, 1922	do [a]	do	.166	.138	+. 028	−20. 25
July, 1923	Cornfield Bog	do	.333	.341	−. 008	−2 35
Do	Vaughn Bog	do	.334	.311	+. 023	+7. 40
Do	Thyonnett Bog	do	.293	.212	+ 081	+38 21

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog

The data for the moisture, lipoid substances, water-soluble substances, and insoluble substances are given in Table 7. Moisture is higher in the healthy stems and leaves. The dry matter is lower in the healthy stems and leaves, the differences being distributed among the three fractions of dry matter.

TABLE 7.—*Moisture, lipoid substances, water-soluble substances, and insoluble substances in tissues of diseased and healthy cranberry plants, 1920 samples* [a]

[Based on fresh weight]

Determination	Part of plant	Percentage			
		In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
Total moisture	Leaves	56. 38	66 36	−9. 98	−15. 03
Lipoid substances	do	10. 73	8. 77	+1 96	+22. 34
Water-soluble substances	do	8 70	6 99	+1. 71	+24. 46
Insoluble substances	do	24. 18	17. 86	+6. 32	+35. 38
Total moisture	Stems	53. 58	55. 44	−1. 86	−3. 35
Lipoid substances	do	5. 10	4. 85	+. 25	+5. 15
Water-soluble substances	do	4. 07	3. 65	+. 42	+11. 50
Insoluble substances	do	37. 22	36. 00	+1. 22	+3. 38

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The amounts of total phosphorus, total ash, water-soluble ash, lipoid ash, and insoluble ash are shown in Table 8. There is no significant difference in the phosphorus content of the healthy and diseased leaves. The healthy stems are slightly higher in phosphorus than the diseased stems. The total ash is highest in the diseased leaves but lower in the diseased stems than in the healthy ones. Water-soluble and insoluble ash were higher in both diseased leaves and stems. The diseased stems and leaves were both lower in lipoid ash.[a]

TABLE 8.—*Total ash, lipoid ash, water-soluble ash, insoluble ash, and total phosphorus content of tissues of diseased and healthy cranberry plants, samples collected in 1920*[a]

[Based on fresh weight]

		Percentage			
Determination	Part of plant	In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with healthy plants	Percentage of difference
Total ash	Leaves	1.400	1 300	+0.10	+7.69
Lipoid ash [b] (F₃)	do	.299	.492	− 193	−39.23
Water-soluble ash (F₁)	do	.218	.151	+ 067	+44.37
Insoluble ash (F₃)	do	.883	.657	+.126	+19 17
Total phosphorus	do	.045	044	+ 001	+2.27
Total ash	Stems	.903	1.210	− 307	−25.37
Lipoid ash [b] (F₂)	do	.207	.639	− 432	−67.60
Water-soluble ash (F₂₁)	do	.286	.198	+.088	+44.44
Insoluble ash (F₂₂)	do	.410	.373	+.037	+9.92
Total phosphorus	do	.047	.056	− 009	−16.07

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.
[b] By difference, total, −(F₂÷₃)

When false blossom of cranberries first became of economic importance in Wisconsin the disease was attributed by Shear (*9, 10*), in part at least, to lack of drainage, whereas Malde (*7*) stated that extreme drought was also responsible in part for the disease. In view of these conflicting opinions it was thought desirable to make a study of the moisture content of some of the bogs on Cape Cod. Samples of soil were collected at various times during the season of 1923 from the State bog, in which there was no false blossom, and from the Vaughn Bog, which was badly infested with the disease. These data are presented in Table 9. Samples of the soil from the State bog were found to be lower in moisture—except those collected on May 26.

Soil samples were also taken from diseased and healthy sections of the same bog at the same time the plants were sampled, in July, 1923. The data are shown in Table 10. This table shows that diseased plants are found on bogs high in moisture and also on those low in moisture content. The soil samples of the diseased section of Vaughn Bog contained 83 per cent moisture, while those from the Thyonnett and Cornfield Bogs contained 36 per cent and 26 per cent, respectively. In two cases out of the three the diseased section of the bog was higher in moisture than the healthy section. These differences, however, are small, varying from 7 to 9 per cent.

Moisture was thought to be an important factor in connection with false blossom because of its supposed effect on the plant's supply of nitrate nitrogen. Therefore nitrate nitrogen was likewise determined on the soil samples collected for the determination of moisture. These data are presented in Tables 9 and 10. Where the moisture is high, as it was in the Vaughn Bog samples, the nitrate nitrogen is in all cases lower. On the other hand, on the Thyonnett Bog, although the moisture is slightly higher in the soil from the diseased section, the nitrate nitrogen is also higher. However, as compared with the quantity of moisture in the Vaughn Bog samples, the moisture of the Thyonnett Bog sample is very low. It is also of interest that no nitrate nitrogen was found in the Cornfield Bog soils.

TABLE 9.—*Moisture and nitrate nitrogen in cranberry-bog soils, 1923*

[The State bog contained healthy plants; Vaughn Bog, diseased plants]

Date of sampling	Percentage of moisture				Milligrams of nitrate nitrogen in 100 grams of soil			
	Vaughn Bog soil	State bog soil	Excess or deficiency in Vaughn Bog soil	Percentage of difference	Vaughn Bog	State bog	Excess or deficiency in Vaughn Bog soil	Percentage of difference
May 26, 1923_____	59 04	76. 54	−17 50	−22. 86	0 049	0. 189	−0.140	−74. 07
June 6, 1923_____	65. 61	52. 88	+12.73	+24 07	.189	.483	−.294	−60 87
June 14, 1923_____	84.35	76 20	+8.15	+10. 69	None	.589	−589	_____
June 21, 1923_____	80. 06	76 04	+4.02	−5. 28				
July 7, 1923_____	82.84				042			
Aug. 15, 1923_____	81.42	71 36	+10 06	+14 09	.217	.636	−.419	−65 88

TABLE 10.—*Moisture and nitrate nitrogen in soil of healthy and diseased sections of cranberry bogs at time of sampling, July, 1923*

Source of material	Percentage of moisture				Milligrams of nitrate nitrogen in 100 grams of soil			
	Diseased-section soil	Healthy-section soil	Excess or deficiency in diseased-section soil as compared with quantity in healthy-section soil	Percentage of difference	Diseased-section soil	Healthy-section soil	Excess or deficiency in diseased-section soil as compared with quantity in healthy-section soil	Percentage of difference
Vaughn Bog_____	82.84	73. 14	+9. 70	+13. 26	0. 042	0. 091	−0 049	−53 84
Thyonnett Bog_____	36.21	28 58	+7. 63	+27. 04	.236	154	+.082	+53 24
Cornfield Bog_____	25.82	33. 02	−7. 20	−21. 80	None.	None.	_____	_____

DISCUSSION

It has been suggested (*10*) that the false-blossom disease of the cranberry is due to a disturbance in the physiological function of the plants, due to an excessive amount of nitrogenous plant-food material. The analyses of diseased and healthy plants presented in this paper do not support this explanation. The diseased plants are much

higher in carbohydrates than the healthy plants, and the total nitrogen of the diseased plants is about equal to that of the healthy. If the disease were due to the presence of large amounts of available nitrogen one would expect to find the diseased plants lower in carbohydrates and higher in total nitrogen than the healthy ones.

Improper cultural conditions (*5*, *9*, *7*), leading to poor drainage of the bog, have also been held responsible for the disease. Determinations of the moisture content of the soil of diseased and healthy sections of the bogs show that diseased plants are found in bogs high in moisture and in those low in moisture. While it is probable that cultural conditions may affect the rate of development of the disease and the extent of damage, the bog-moisture data indicate that improper cultural conditions are not the cause of the disease.

From observations (*11*) of the spreading of the disease to Massachusetts and Oregon from Wisconsin through nursery stock, considerable evidence has been presented to show that the disease is infectious. It is probable that the disease may be similar to the virus diseases such as the yellows and the mosaic diseases. It is interesting from this standpoint to compare the composition of healthy and diseased cranberry tissues with those of the healthy and diseased mosaic plants. The very much larger carbohydrate content of the diseased plants is similar to that found in spinach mosaic by True and Hawkins (*12*). No consistent difference was found in the total nitrogen content of the diseased and healthy cranberry plants. Jodidi, Moulton, and Markley (*4*) found the total nitrogen content (oven-dry basis) of the mosaic spinach plant consistently lower than the healthy The healthy cranberry plant differs from the healthy spinach plant, however, in containing a much smaller amount of nitrogen. The spinach plant contains approximately twice as much nitrogen as the cranberry plant. The nonprotein nitrogen of the cranberry plant is exceptionally low.

True, Black, and Kelly (*13*) found the above-ground portions of the healthy spinach plants higher in ash on the dry-weight basis than the diseased. The sum of the ash contents of the cranberry leaves and stems divided by two gives a figure which represents fairly accurately the amount of ash in the entire plant and may be compared with the data on the spinach plant. Recalculating the results obtained upon this basis, it is found that the above-ground portions of the healthy cranberry plants are slightly higher in ash content than the diseased, on the fresh-weight basis, and much higher than the diseased, on the dry-weight basis.

Hartwell (*3*) has pointed out that the lack of sufficient available nitrogen, phosphorus, or available moisture or other conditions which retard growth lead to the accumulation of starch in the above-ground portions of the plant. It has been pointed out, however, that the disease is not due to lack of sufficient available nitrogen or to lack of sufficient moisture. The analyses show no significant difference in phosphorus content of diseased and healthy cranberry tissues. Moreover, numerous fertilizer experiments have shown that the disease can not be prevented by the application of mineral nutrients. In view of these facts it seems very probable that the false-blossom disease of the cranberry may be a virus disease similar to the yellows and mosaic diseases.

SUMMARY

Cranberry plants having the disease false blossom are higher in free reducing sugars, sucrose, starch, acid-hydrolyzable substances, and dry matter and lower in moisture than healthy plants.

No consistent differences were found in the nitrogen content of diseased and healthy plants.

The ash content of the tops of healthy plants is slightly higher than that of the diseased plants.

False blossom of cranberries occurs under widely varying conditions of soil moisture. Improper drainage conditions are not the cause of the disease.

The disease is not caused by excessive quantities of available nitrogen.

It is suggested, as the result of analyses of healthy and diseased plants, that the false-blossom disease of the cranberry may be a virus disease similar to the yellows and mosaic diseases.

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THE NITROGEN METABOLISM OF STEERS ON RATIONS CONTAINING ALFALFA AS THE SOLE SOURCE OF THE NITROGEN [1]

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INTRODUCTION

In 1923 the National Research Council's subcommittee on animal nutrition published a list of problems in the field of animal nutrition [2] which they recommended as worthy, in their opinion, of extensive and thoroughgoing study. One of these problems was that of estimating metabolic nitrogen.

The committee proposed the following procedure:

Choose several proteins which, presumably, are completely digestible. In nitrogen balance experiments add each of these proteins in graduated amounts, in a series of experimental periods, to otherwise nitrogen-free basal rations. That kind of protein which, when thus varied in amount, does not cause the total feces nitrogen to vary we would rate as *completely digestible;* and the total feces nitrogen in these periods would be considered as of metabolic origin.

Shortly before these recommendations were published the writer had prepared plans for a study of the mutual influence of the proportion of the several nutrients in feeds on their digestibility.[3] The procedure outlined was as follows: A 12-pound ration of alfalfa was to be fed to steers for a period of at least two weeks and then two digestion trials, with an interval of at least one week between them, were to be conducted to determine the digestibility of this ration. Then, in place of a part of the original ration, an equal weight of one of the pure nutrients (as nearly pure as it could be obtained) was to be substituted. This modified ration was to be fed for a period of two weeks and then two digestion trials were to be conducted as before. Again, in place of a larger part (twice as large as before) of the ration, an equal weight of the pure nutrient was to be substituted and, after a preliminary two weeks of feeding, two more digestion trials were to be conducted. This modifying of the original ration was to be done four times.

It was planned to study first the effect of crude fiber. As a source of relatively pure crude fiber filter-paper pulp was chosen. Inasmuch as this filter-paper pulp was practically nitrogen free, it will be seen that the writer's procedure was essentially a reversal of the plan recommended by the subcommittee on animal nutrition, in that instead of adding protein to a nitrogen-free ration nitrogen-free material was substituted for an equal weight of a ration relatively rich in protein.

Influenced in part by the recommendations of the subcommittee on animal nutrition, the writer decided to analyze the data obtained

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 1
Jan. 1, 1927
Key No. N. Mex.-2

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to see if they would throw some light on the problem of estimating metabolic nitrogen and on the general problem of nitrogen metabolism. Accordingly, during the course of the investigation and afterwards, the data were so analyzed. The results of this analysis are given in this paper.

EXPERIMENTAL PROCEDURE

The general plan of the investigation has already been given. Five "long yearling" steers [4] were fed for a period of 226 days, and during that time a series of nine digestion trials was conducted with them. Four of the five steers were used in each of the first two digestion trials and three [5] were used in each of the last seven. The digestion trials were 10 days in length and the intervals between them were (a) 11 days between digestion trials on the same ration and (b) 18 days between digestion trials on different rations. It was during these nine digestion trials that the nitrogen metabolism data herein recorded were secured.

The weight of the feed given daily to each steer was 12 pounds,[5] one-half of this amount at 5 p. m.[6] and the other half at 6.30 a. m. The steers were watered at 3 p. m. and at 10 a. m. Samples of urine and feces were taken each day at 12 m.

Complete details of the investigation from which the data herein given were obtained have been published elsewhere.[7]

COMPOSITION OF THE COMPONENTS OF THE RATIONS, AND OF THE RATIONS

Five different rations were fed; one consisted solely of alfalfa, the other four were definite mixtures of alfalfa and paper pulp. The percentage of each of the two components in each of the five rations was as follows:

	Ration No. 1.	Ration No. 2.	Ration No. 3.	Ration No. 4.	Ration No. 5.
Alfalfa (per cent)	100	85	70	55	40
Paper pulp (per cent)	0	15	30	45	60

The first cutting of alfalfa of very good quality was employed throughout the investigation. The paper pulp used was a high-grade whole-rag commercial product. The composition of the dry matter of the alfalfa and paper pulp is given in Table 1. The composition of the feed used in each of the nine digestion trials is given in Table 2.

TABLE 1.—*Composition of the dry matter of the alfalfa and paper pulp*

ALFALFA

Statistical constants	Ash	Protein	Non-protein	Crude fiber	Ether extract	Nitro-gen-free extract	Total nitro-gen	Protein nitro-gen
	P. ct.	*P. ct.*	*P. ct.*	*P. ct*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Mean [a]	10. 97	14. 03	2. 12	32. 78	1. 62	38. 48	2. 694	2. 244
Probable error of mean	±. 03	±. 06	±. 02	±. 10	±. 01	±. 08	±. 009	±. 009
Standard deviation	. 19	. 40	. 14	. 70	. 09	. 55	. 064	. 065
Coefficient of variation	1. 76	2. 88	6. 74	2. 15	5. 49	1. 42	2. 39	2. 88

PAPER PULP

Mean [b]	0. 94	0. 19	Trace	87. 12	0. 10	11. 64	0. 030	0. 028
Probable error of mean	±. 03	±. 02		±. 25	±. 01	±. 25	±. 002	±. 002
Standard deviation	. 20	. 09		1. 46	. 04	1. 44	. 009	. 010
Coefficient of variation	21. 08	46. 02		1. 67	35. 87	12. 37	29. 11	46. 02

[a] Computed from the results of 22 separate analyses; the average moisture content of the 22 samples was 8 44 per cent.
[b] Computed from the results of 15 separate analyses; the average moisture content of the 15 samples was 5.67 per cent.

TABLE 2.—*Composition of the feed used in each of the nine digestion trials* [a]

Ra-tion No.	Diges-tion trial No	Mois-ture	Ash	Protein	Non-protein	Crude fiber	Ether extract	Nitro-gen free extract	Total nitro-gen	Protein nitro-gen
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	1	8. 33	10. 25	13. 64	1. 84	29. 68	1. 59	34. 67	2. 573	2. 182
	2	8. 85	9. 80	12. 71	2. 02	29. 84	1. 54	35. 24	2. 464	2. 034
2	3	8. 32	8. 64	10. 63	1. 63	38. 06	1. 26	31. 46	2. 047	1. 701
	4	9. 76	8. 17	10. 26	1. 55	38. 52	1. 17	30. 57	1. 971	1. 642
3	5	8. 32	6 99	8. 38	1 55	46. 61	. 92	27. 23	1. 672	1. 341
	6	7. 15	7. 05	8. 55	1. 38	47. 07	. 96	27. 84	1. 660	1. 368
4	7	6. 57	5. 90	6. 80	1. 05	55. 50	. 67	23. 51	1. 310	1. 088
	8	5. 44	6. 01	7. 21	1. 10	55. 86	. 67	23. 71	1. 386	1. 152
5	9	4. 87	4. 71	5. 06	. 79	63. 90	. 45	20. 22	. 977	. 810

[a] As fed.

THE NITROGEN DATA

Because of the many data involved, only average values of these which pertain to the nitrogen metabolism will be given here. The nitrogen content of the rations and the average nitrogen content of the total excreta, the feces, and the urine are given in Table 3; these same data are presented graphically in Figure 1.

TABLE 3.—*Nitrogen metabolism data (10 days)*

Ra-tion No.	Diges-tion trial No.	Num-ber of steers aver-aged	Nitrogen in the feed	Average amount of nitrogen in the total excreta	Average amount of nitrogen in the feces		Average amount of nitro-gen in the urine	Average amount of nitro-gen re-tained
					Found	Corrected value [a]		
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	1	4	1,400. 51	1,379. 77	388. 00	392. 95	991. 77	20. 74
	2	4	1,331 77	1,331. 25	377. 60	372. 79	953. 65	. 52
2	3	3 ·	1,114. 20	1,085. 26	350. 01	350. 85	735. 26	28. 93
	4	3	1,072. 84	1,049. 98	346. 99	334. 51	702. 99	22. 86
3	5	3	910. 09	833. 36	316. 72	313. 94	516. 64	76. 73
	6	3	903. 56	839. 90	332. 73	310. 87	507. 17	63. 66
4	7	2	713. 05	624. 45	340. 74	297. 01	283. 71	88. 60
	8	2	754. 41	658. 56	340. 35	303. 25	318. 21	95. 85
5	9	2	508. 19	483. 96	313. 07	256. 85	170. 89	24. 23

[a] Corrected to a uniform water content of 80 per cent by the use of the factor 0.001704 given in equation (1)

METABOLIC NITROGEN

In order to see if the nitrogen content of the feces varied uniformly with the amount of nitrogen ingested, the weight of nitrogen in the feed (for 10 days) was plotted against the average weight of the nitrogen in the feces (for 10 days).   (Fig. 1.)   All the resulting points did not lie on a straight line; however, deviations from a straight line were concomitant with a high water content of the feces.   For this
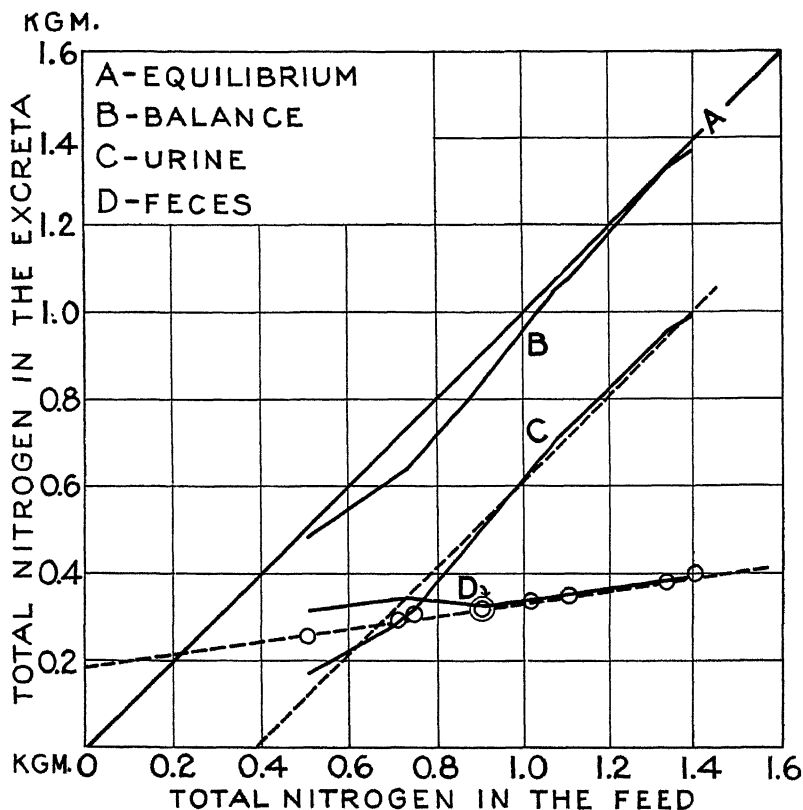
FIG. 1.—Nitrogen metabolism data for 10-day periods. A is the line of nitrogen equilibrium. B is the nitrogen balance curve, that is, the nitrogen in the feed plotted against the nitrogen in the total excreta; the vertical distance between A and B represents the amount of nitrogen retained, if B is below A, and the amount of nitrogen lost, if B is above A. The solid line C results from plotting the nitrogen in the urine against the nitrogen in the feed; the broken line C is a straight line fitted to these plotted points. The solid line D results from plotting the nitrogen in the feces against the nitrogen in the feed; the circles are for the same set of data *after* correcting the nitrogen content of the feces to a uniform water content (80 per cent) of the feces; the broken line D is a straight line fitted to the plotted points indicated by the circles

reason it seemed that the nitrogen content of the feces might be influenced by the water content of the feces.   The fact that the above-mentioned points did give a straight line for the first six digestion trials and the additional fact that the slope of this line was much less than would have been observed if the nitrogen in the feed had had the same apparent digestibility throughout, indicated that for each ration there was a more or less fixed amount of nitrogen (metabolic) in the feces which was independent of the true digestibility of the nitrogen of the

feed. Such an amount of nitrogen in the feces would cause the apparent digestibility of the feed nitrogen to become less and less as the amount of ingested feed nitrogen is decreased.

Pfeiffer [8] corrected for the nitrogenous excretory products by subtracting 0.4 gm. of nitrogen per 100 gm. of digested dry matter. Assuming that the quantity of metabolic nitrogen in the feces is dependent upon the amount of digested dry matter and upon the amount of water in the feces, an attempt was made to determine the empirical relationship. As a result of this attempt, the following equation for determining the amount of metabolic nitrogen in the feces was obtained:

$$m = 0.002813a + 0.001704b \tag{1}$$

in which

m = weight of metabolic nitrogen in the feces,
a = weight of dry matter digested, and
b = weight of water in the feces.[9]

If it is assumed that the metabolic nitrogen in the feces is dependent upon the dry matter consumed and the water content of the feces, rather than upon the dry matter digested and the water content of the feces, the following relationship is obtained:

$$m = 0.002428a_1 + 0.001562b \tag{2}$$

in which

m = weight of metabolic nitrogen in the feces,
a_1 = weight of dry matter consumed, and
b = weight of water in the feces.

When the average digestibility of the nitrogen in the alfalfa is computed, after correcting for metabolic nitrogen, it is found to be 88.16 per cent if correction is made according to equation (1), and 90.13 per cent if correction is made according to equation (2).

It can not be said, as yet, which equation will give more nearly the correct amount of metabolic nitrogen in the feces. Application of the two equations and further investigation should give some indication as to which set of assumptions is more nearly correct and to what extent the equation dependent upon that set of assumptions may be relied upon. The writer gives preference to equation (1).

As a check on the previously mentioned indication that the water content of the feces influences its nitrogen content, the nitrogen content of the feces was corrected [10] to a uniform water content of 80 per cent. The resulting values were plotted against the nitrogen

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[8] According to Armsby. ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 120. New York. 1917.
[9] In determining the constants in equation (1), an equation similar to the following was set up:

$$ax + by - nz + d = 0$$

in which
    a = weight of the dry matter digested from the feed,
    x = weight of metabolic nitrogen dependent upon unit weight of digested dry matter,
    b = weight of water in the feces,
    y = weight of metabolic nitrogen dependent upon unit weight of water in the feces,
    n = weight of total nitrogen in the feed eaten,
    z = the "true" digestibility of the feed nitrogen, and
    d = the difference between the weight of feed nitrogen and the weight of fecal nitrogen.
This equation, then, expresses the following relationship: The nitrogen not excreted in the feces (d) is equal to the total amount of nitrogen digested (nz) minus the metabolic nitrogen ($ax + by$).

The proper values of a, b, n, and d (for each digestion trial) were then substituted in this equation; thus, nine equations in the three unknowns were obtained. The values of x, y, and z were then determined by the method of least squares. They were found to be 0.002813, 0.001704, and 0.8816, respectively.

The values of the constants in equation (2) were obtained in a similar manner.
[10] By the use of the factor 0.001704, given in equation (1).

content of the feed and all the points (indicated by circles in fig. 1) were found to lie very nearly along a straight line. This would indicate that the water content of the feces does influence its nitrogen content.

The equation of this straight line, determined by the method of least squares,[11] was found to be:

$$y = 0.1447x + 184.95 \tag{3}$$

in which

$y =$ weight (gms.) of nitrogen in the feces (10 days) after being corrected to an 80 per cent water content, and

$x =$ weight (gms.) of nitrogen in the feed (10 days).

This line intersects the y-axis at 184.95 and hence this value is the weight of metabolic nitrogen which the feces (for 10 days) would contain if the ration (12 pounds) contained only paper pulp, i. e., if the ration were nitrogen-free. This is appreciably less than the amount of metabolic nitrogen (219.14 gm.—average value) estimated as resulting from the ingestion of a 12-pound ration of alfalfa. The conclusion follows that the amount of nitrogen in the feces of a steer consuming a nitrogen-free ration may not safely be taken as a measure of the amount of metabolic nitrogen resulting from the ingestion of an equal weight of alfalfa, or other feeding stuff.

THE "TRUE" DIGESTIBILITY OF THE NITROGEN IN ALFALFA

If we correct the nitrogen content of the feces, by subtracting the amounts of metabolic nitrogen estimated by the use of equation (1), and then compute the average "true" digestibility, we find, as stated before, that it is 88.16 per cent. The average "true" digestibility of nitrogen, for each digestion trial, is as follows:

Trial No. 1	Trial No. 2	Trial No. 3	Trial No. 4	Trial No. 5	Trial No. 6	Trial No. 7	Trial No. 8	Trial No. 9
87.62	88.45	87.99	88.65	88.43	88.88	87.65	87.73	88.03

The mean value is 88.16; the probable error of the mean is ± 0.10; the standard deviation is ± 0.43; and the coefficient of variation is 0.49 per cent.

Realizing that the above method of calculating the extent of the variation (i. e., by using average values) has a tendency to decrease the probable error of the mean, the standard deviation, and the coefficient of variation, the "true" digestibility of the nitrogen in the ration of each steer during each digestion trial was computed and, from these 29 values, the above mentioned statistical constants were recalculated. The following values were thus obtained: Probable error of the mean, ± 0.39; standard deviation, ± 2.03; and coefficient of variation, 2.31 per cent. The greatest variations were observed during the last three digestion trials; for the first six trials the mean (of 20 values) was 88.31, the probable error of the mean was ± 0.16, the standard deviation was ± 1.08, and the coefficient of variation was 1.22 per cent.

Thus it is seen that rather concordant values of the "true" digestibility of the nitrogen in alfalfa were obtained when correction was made by the use of equation (1).

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[11] The average values were weighted according to the number of steers used in securing them.

RELATION BETWEEN NITROGEN CONTENT OF THE URINE AND NITROGEN CONTENT OF THE TOTAL EXCRETA

When the nitrogen content of the urine was plotted against the nitrogen content of the total excreta, all the resulting points lay very nearly upon a straight line. The equation of this straight line was determined by the method of least squares [11] and found to be:

$$y = 0.9171x - 266.56 \qquad (4)$$

in which:

y = weight (grams) of nitrogen in the urine (10 days), and

x = weight (grams) of nitrogen in the total excreta (10 days).

It should be noted that equation (4) holds only when the weight of the ration is 12 pounds. When the weight is not 12 pounds, the constant 266.56 will have a different value. For other weights the following equation is tentatively suggested:

$$y = 0.9171x - 22.21R \qquad (4a)$$

in which R is the weight of the ration in pounds.

As a check on the constancy of this relationship the nitrogen content of the urine was calculated, by means of equation (4), from the corresponding nitrogen content of the total excreta. These calculated values, together with the average values found and the difference between the found and calculated values, are given in Table 4. The found and calculated nitrogen content of the urine of the individual steers when consuming a 12-pound ration of alfalfa is given in Table 5. The indications are that this relationship between the nitrogen content of the urine and the nitrogen content of the total excreta is very constant when alfalfa is the source of the nitrogen.

TABLE 4.—*The found and calculated nitrogen content of the urine (10 days), by digestion trials*

Ration No.	Digestion trial No.	Number of steers averaged	Average weight of the nitrogen in the urine			
			Found	Calculated	Absolute difference	Proportional difference
			Gm.	*Gm.*	*Gm.*	*Per cent*
1	1	4	991.77	998.83	−7.06	0.71
	2	4	953.85	954.51	−.66	.07
2	3	3	735.26	728.74	+6.52	.89
	4	3	702.99	696.38	+6.61	.95
3	5	3	516.64	497.71	+18.93	3.80
	6	3	507.17	503.71	+3.46	.69
4	7	2	283.71	306.12	−22.41	¯.32
	8	2	318.21	337.41	−19.20	5.69
5	9	2	170.89	177.08	−6.19	3.50

[11] The average values were weighted according to the number of steers used in securing them.

TABLE 5.—*The found and calculated nitrogen content of the urine (10 days) of the individual steers when consuming a 12-pound ration of alfalfa*

Diges-tion trial No.	Steer No	Weight of the nitrogen in the urine			
		Found	Calcu-lated	Absolute differ-ence	Propor-tional differ-ence
		Gm.	*Gm.*	*Gm.*	*Per cent*
1	42	961.31	985.66	−24.35	2.47
	43	966.96	976.18	−9.22	.95
	44	1,023.27	1,022.72	+.55	.05
	45	1,015.53	1,010.75	+4.78	.47
2	42	924.86	950.40	−25.54	2.69
	43	960.91	966.29	−5 38	.56
	44	957.89	948.70	+9.19	.97
	45	970.96	951.92	+19.04	2.00
Average					1 27

URINARY NITROGEN AND THE NITROGEN REQUIREMENT FOR MAINTENANCE

When the average nitrogen content of the urine (for 10 days) was plotted against the nitrogen content of the feed, the resulting points (fig. 1) indicated a more or less linear relationship. The equation of the straight line fitted to these points was found to be:

$$y = 0.9937x - 383.69 \qquad (5)$$

in which

$y =$ weight (gms.) of nitrogen in the urine (10 days), and
$x =$ weight (gms.) of nitrogen in the feed (10 days).

This equation represents, at least approximately, the manner in which the urinary nitrogen decreased as the feed nitrogen was decreased. The x-intercept [12] of the line drawn according to the above equation is 386.14. However, a steer consuming 386.14 gm. of nitrogen in its feed, per 10 days, would excrete an appreciable amount of nitrogen in its urine, and so it is clear that equation (5) can not represent conditions when the nitrogen intake approaches and becomes less than that required for maintenance.

Had this investigation been conducted with mature steers, it seems possible that the x-intercept of this line [13] might represent the animals nitrogen requirement for maintenance. If, in this case, the x-intercept be taken as the average amount of nitrogen required for the maintenance of each steer during the last three digestion trials, we find that 386.14 gm. of nitrogen was required to maintain a 780-pound steer (average weight) for 10 days; this is equivalent to 0.493 pound of digestible[14] crude protein, per day, per 1,000 pounds average live weight. This value is distinctly lower than the value given by Armsby,[15] but within the range of variation he suggested for his value.

[12] The point on the line corresponding to a urinary nitrogen excretion of zero.
[13] The points fixing this line would have to be determined by nitrogen intakes well above the maintenance requirements.
[14] *Apparent* digestibility, 71.97 per cent.
[15] ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 327. New York. 1917.

THE NITROGEN BALANCES

In Figure 1 the nitrogen in the feed was plotted against the nitrogen in the total excreta and the line connecting the resulting points was called a "balance curve." The amount of nitrogen retained is represented by the vertical distance between the balance curve and the equilibrium line.

One point worthy of observation is that the nitrogen balances became larger and larger as the amount of paper pulp in the ration increased [16] until it amounted to 45 per cent; after that the nitrogen balances decreased. The only explanation offered (and it does not seem wholly satisfactory) is that the available energy of the ration was the limiting factor during the first part of the investigation and that during the latter part (when the ration contained only 40 per cent alfalfa) the nitrogen of the ration was the limiting factor. This explanation involves the assumption that, weight for weight, the paper pulp supplied an appreciably greater amount of available energy than did the alfalfa. In view of the high apparent digestibility (77.56 per cent [17]) of the paper pulp, the above assumption could easily be true.

CONCLUSIONS

The results of the analysis of the data given in this paper seem to warrant the following conclusions:

(1) The amount of metabolic nitrogen in the feces of a steer is influenced, among other things, by the water content of the feces.

(2) A linear relationship exists between the nitrogen content of the feces of a steer (when corrected to a uniform water content) and the nitrogen content of its feed, when the latter is decreased by substituting paper pulp (probably any digestible carbohydrate material) for an equal weight of a given ration of alfalfa.

(3) The fecal nitrogen excretion of a steer consuming a nitrogen-free ration can not safely be taken as a measure of the metabolic nitrogen in the feces of the animal when consuming an equal weight of a given feeding stuff.

(4) The "true" digestibility of the nitrogen of alfalfa is approximately 88 per cent.

(5) A linear relationship exists between the nitrogen content of the urine and the nitrogen content of the total excreta of a steer, if the source of the nitrogen is alfalfa, when the nitrogen content of the feed is varied as it was in this investigation.

(6) When the nitrogen in the feed is varied, as it was in this investigation, an approximately linear relationship exists between the nitrogen content of the urine and the nitrogen content of the feed, if the animal, at all times, is receiving appreciably more nitrogen than it requires for maintenance. With a mature steer (possibly with a

[16] This is similar to the experience of Wicke and Weiske, who added 174 gm. of starch to a basal ration containing 800 gm. of hay and 200 gm. of flaxseed and observed an increase of the nitrogen balance (with sheep) from 0.96 gm. to 1.64 gm. per day. WICKE, A., and WEISKE, H. ÜBER DEN EINFLUSS EINER FETT-RESP. STARKEBEIGABE AUF DIE AUSNÜTZUNG DER NÄHRSTOFFE IM FUTTER UND AUF DEN N-UMSATZ UND ANSATZ IM THIERKÖRPER. (2. VERSUCHSREIHE.) Hoppe-Seyler's Ztschr. Physiol. Chem. 22: 137–152. 1896.

[17] TITUS, H. W. THE MUTUAL INFLUENCE OF THE PROPORTION OF THE SEVERAL NUTRIENTS, IN FEEDS, ON THEIR DIGESTIBILITY. I. CRUDE FIBER—THE DIGESTIBILITY OF RATIONS CONTAINING VARYING AMOUNTS OF ALFALFA AND PAPER PULP. N. Mex. Agr. Expt. Sta. Bul. 153, 52 p., illus. 1926.

growing steer) this relationship may be of value in estimating the amount of nitrogen the steer requires for maintenance.

(7) The substitution of paper pulp (within certain limits) for an equal weight of alfalfa, in a ration consisting solely of alfalfa, causes an increased retention of nitrogen. It is highly probable that this effect of the paper pulp is dependent upon the size of the original alfalfa ration.

INJURY TO GROWING CROPS CAUSED BY THE APPLICATION OF ARSENICAL COMPOUNDS TO THE SOIL[1]

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INTRODUCTION

In a preliminary paper (*14*)[2] brief mention was made of the report of Headden (*8*) on the injury to crowns of fruit trees in Colorado caused by the use of arsenicals, and a detailed outline was given of a series of experiments made by the writers for the purpose of determining whether the arsenical compounds commonly used as insecticides are injurious to the bark of fruit trees, the nature and extent of injury, the relative toxicity of different arsenicals, and the conditions most favorable to injurious action.

The work here recorded, which was done between 1911 and 1922, at Bozeman, Mont., and at Madison, Wis., presents another phase of the general problem of the effect of arsenical compounds on plant life, but deals primarily with the results obtained by adding the chemical to the soil.

HISTORICAL REVIEW

A review of early literature reveals the fact that arsenical compounds, especially arsenic trioxide, were known in ancient times. Arsenic was early recognized as a typical poison for plants as well as animals, and its effects upon plant life have been the subject of investigation for many years.

De Candolle (*3, p. 1328–1332*) in 1832 refers to the work of Jaeger, Marcet, and Macaire and reviews in considerable detail the work of Leuchs, who found a decrease in dry weight of vetch plants when grown in the presence of arsenic trioxide.

Chatin (*4*) discovered that arsenic absorbed by the roots was distributed to various tissues of plants.

Davy (*1*) concluded from his work on the effect of arsenic on growing plants: (1) That plants, at least peas and turnips, are not injured by arsenic in the soil; (2) that they absorb this dangerous element; and (3) that they retain it in their tissues.

Daubeny (*5*) watered barley plants five times in succession with a solution of arsenious acid, 1 ounce in 10 gallons of water, and found that the crop matured a fortnight earlier, but that the amount of grain harvested was less than the normal yield. Four waterings of turnips with the same solution did not hasten maturity, but slightly decreased the yields. Analysis did not indicate any arsenic in the tissues.

Gorup (*7*) grew to maturity .plants of *Polygonum fagopyrum*, *Pisum sativum*, and *Secale sativum* in soil containing arsenious acid, 30 gm. of the acid in 30.7 cu. decim. of soil, each unit growing two plants which matured normally. Analysis by Marsh's test showed

no trace of arsenic in 20 gm. of dry matter of *Secale cereale*, but in 148
gm. of *Polygonum fagopyrum* a weak mirror was formed.   He con-
cluded that these plants, especially *Pisum sativum*, are indifferent to
relatively large quantities of arsenious acid in the soil.

Freytag (*6*) found that one-eightieth per cent of arsenious acid in
water culture solution was fatal to beans, peas, and other plants, and
he concluded that plants have not a selective power since they absorb
poisonous as well as nutritive substances.

McMurtie (*10*) concluded from his work that plants have not the
power to absorb and assimilate compounds of arsenic from the soil
but that such compounds may exert an injurious influence upon
vegetation, although not until the quantity present reaches in the
case of Paris green about 900 pounds per acre, in the case of arsenite
of potash about 400 pounds per acre, and in the case of arsenate of
potash about 150 pounds per acre.

Phillips (*12*), experimenting with greenhouse plants, found that
calcium arsenate when present in the soil in toxic amounts checked the
formation of roots to such an extent as to interfere with nutrition
and growth, or else it killed the plant outright.   Analyses showed
no traces of arsenic in the poisoned plants.

Nobbe, Baessler, and Will (*11*) found that one part of arsenic in
one million parts of water had an injurious effect on buckwheat, oats,
maize, and peas when grown in water culture.

Blyth (*2*) stated that if plants are poisoned with arsenic, the toxic
action may be traced from below upwards, and analyses will detect
minute quantities of arsenic in all parts of the plant.

Lyttkens (*9*) found that the addition of 0.005 to 0.01 per cent of
arsenious acid (as the potassium salt) to garden soil in which barley
was growing caused a feeble growth and a blue-green color.

In regard to the occurrence of arsenic in plant tissues, a great deal
of work has been done, and the general conclusion has been reached
that arsenic is quite generally a constituent of plant tissue when
plants are grown in its presence.

Wanklyn (*16*), discussing arsenic, says that "minute traces of
arsenic are all-pervading, and, as necessary consequence, the mere
detection of arsenic is devoid of meaning unless it is, to some extent,
a quantitative operation."

DESCRIPTION AND RESULTS OF EXPERIMENTS

TRANSPIRATION STUDIES WITH OAT PLANTS IN WATER CULTURES

A group of preliminary experiments was made with oat plants in
water cultures.

The containers used were ordinary wide-mouthed flint glass bottles
of approximately 530 c. c. capacity, covered with a uniform coat of
varnish.   They were cleaned with a sulphuric-potassium-chromate
cleaning fluid and thoroughly rinsed with distilled water.   The
water was obtained by double distillation; its conductivity, tested
at different times, varied from 4 to 6 times 10^{-5} ohms.   The chemicals
of a high degree of purity, were as follows: Arsenic trioxide, by
analysis 73.1 per cent arsenic; calcium nitrate; ferric chloride; mag-
nesium sulphate; potassium phosphate (monobasic); Shive's (*13*)
3-salt nutrient solution No. R5C2, which contained potassium phos-
phate, 0.018 m., calcium nitrate, 0.0052 m., magnesium sulphate,

0.0150 m; and ferric chloride 0.068 per cent solution, 5 c. c. to each 500 c. c. of nutrient solution.

Oat plants (Foundation Wisconsin Wonder Stock Pedigree No. 1) were used in all experiments. These were germinated in moistened pure quartz sand, and the seedlings were supported over the nutrient solution by the method described by Tottingham (*15*). When arsenic trioxide was used in the solution, it was added at the rate of a definite number of parts per million calculated as metallic arsenic.

The loss of weight by transpiration was determined at the end of each week by weighing the cultures, and enough distilled water was added at each weighing to compensate for the loss by transpiration. In some of the experiments the plants were severed just above the remains of the seeds, and the tops were dried at a constant temperature of 102° C. until they became constant in weight. The results of these experiments are recorded in Tables 1, 2, and 3.

TABLE 1.—*Loss of water by oat plants grown in Shive's nutrient solution with and without the addition of arsenic trioxide*

Culture No.	Arsenic, parts per million	Water lost (grams)				Appearance of plants
		First week	Second week	Third week	Total	
1	0	9.5	23.5	35 5	68.5	}Normal color, leaf blade broad.
2	0	10 0	23.0	36 5	69.5	
3	1	10.0	19.0	20.5	49.5	Light-green color, leaf blades narrower than Nos. 1 and 2.
4	1	9.0	17.5	19.0	45.5	
5	3	6.5	12.0	14.0	32.5	Lighter colored, and leaf blades narrower than Nos. 3 and 4.
6	3	6.0	ª 9.5	10.5	ᵇ 26	

ª One plant made no growth, probably because of fungous attack.
ᵇ No allowance is made for dwarfed plant.

TABLE 2.—*Loss of water by oat plants grown in Shive's nutrient solution, with and without the addition of arsenic trioxide*

Culture No.	Arsenic, parts per million	Water lost (grams)					Dry weight of tops in grams	Appearance of plants
		First week	Second week	Third week	Fourth week	Total		
1	0	3.5	10.0	22.5	48.5	84 5	0.1990	}Leaf blades broad, normal color, average height 400 mm. Average length roots 85 mm.
2	0	1.5	8.5	23.5	44 5	78.0	.1820	
3	0	3.0	10.0	24.5	50 0	87.5	.1974	
4	5	2 0	ª 6.0	6.0	9 5	ᵇ 23.5	ᵇ.0630	}Leaf blades slightly rolled, light-green color, average height 200 mm. Average length roots 25 mm.
5	5	2 5	4.5	4.5	12.0	23.5	ª.0622	
6	5	1.0	4.0	7.0	11.5	23.5	.0854	
7	10.	1.5	3.5	5.0	13.0	23.0	.0778	}Color light green. Average height 165 mm. Average length roots 25 mm.
8	10	1.5	ª 3.0	4.5	9.0	ᵇ 18.0	ᵇ.0591	
9	10	1.0	2.5	4.5	11.5	19.5	.0614	

ª One plant dead.
ᵇ No allowance is made for dead plant.

TABLE 3.—*Loss of water by oat plants grown in Shive's nutrient solution, with and without the addition of arsenic trioxide*

Culture No.	Arsenic, parts per million	Water lost (grams)				Dry weight tops (grams)	Appearance of plants
		First week	Second week	Third week	Total		
1	0	12.0	31.0	56.0	99.0	0.3270	Good color, root system well developed; leaf blades broad.
2	0	8.5	30.5	58.5	97.5	.3160	
3	0	10.0	29.5	55 0	94.5	.3300	
4	5	7.0	17 0	13.5	37.5	.1212	Light-green color, root system poorly developed; leaf blades narrow.
5	5	6 5	13.5	12 0	32.0	.1090	
6	5	9 5	15.5	17.0	42 0	.0936	
7	10	5 0	8 0	8.5	21.5	.0682	Similar to 4, 5, and 6, but all effects more noticeable.
8	10	4.5	5.0	2.5	12 0	.0628	
9	10	8.5	9 0	9.5	27 0	.0694	

Tables 1, 2, and 3 show rather conclusively that arsenic added as arsenic trioxide decreases transpiration, even when added at the rate of one part per million. The treated plants were characterized by a lighter-green foliage and narrower leaf blades than the control.

While all plants were treated similarly, the results indicate considerable individuality in the plants themselves.

TRANSPIRATION STUDIES WITH TOMATO PLANTS POTTED IN SOIL

Tomato plants were given the ordinary cultural care of a well-managed greenhouse until the plants were about 8 inches tall and of a size suitable for transpiration work. Throughout the experiments all plants were kept as nearly as possible under similar conditions of light, temperature, and humidity.

In determining the amount of water lost by transpiration the soil and pot were so sealed as to allow no loss of water except through the aerial parts. A quantity of water equal to that lost by the plants was added daily, all losses being determined by weighing on scales sensitive to 0.5 gm.

In beginning an experiment, transpiration records were taken for a period of days, usually six, and the plants were then divided into groups of three each. One of these groups was retained as a control, and to the others a single application of arsenic trioxide in solution was added in different proportions after weighing the plants on the sixth day, and the experiments continued. The arsenic trioxide was added as a definite number of parts per million of metallic arsenic based upon the amount of moisture in the soil. The results of these experiments and of similar experiments with sodium arsenite and potassium arsenite are shown in Tables 4 to 7 and in Figures 1 to 4.

TABLE 4.—*Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of arsenic trioxide, the chemical added after the plants were weighed on the sixth day*

SERIES 1

Day	Loss of water in grams by control				Loss of water in grams after addition of—											
					Arsenic 25 parts per million				Arsenic 37 5 parts per million				Arsenic 50 parts per million			
	a	b	c	Total	a	b	c	Total	a	b	c	Total	a	b	c	Total
1	41	39	29	109	34	29	44	107	33	31	44	108	35	26	51	112
2	31	29	23	83	24	20	31	75	23	25	29	77	24	20	32	76
3	60	63	48	171	44	41	79	164	51	46	63	160	48	40	63	151
4	48	45	33	126	41	34	54	129	37	38	48	123	47	36	49	132
5	72	66	49	187	51	46	83	180	63	53	71	187	59	49	71	179
6	67	54	37	158	53	41	66	160	49	44	62	155	64	50	55	169
7	68	76	54	198	46	50	82	178	62	46	71	179	53	44	55	152
8	61	56	44	161	45	43	58	146	44	38	50	132	53	39	46	138
9	88	91	72	251	68	63	102	233	65	59	77	201	83	66	68	217
10	106	100	72	278	71	71	103	245	80	69	93	242	87	72	81	240
11	112	93	70	275	65	70	101	236	90	61	88	239	77	68	76	221
12	80	63	49	192	45	47	68	160	58	44	55	157	58	48	53	159

SERIES 2

Day	a	b	c	Total	a	b	c	Total	a	b	c	Total	a	b	c	Total
1	45	22	39	106	29	42	49	120	33	31	47	111	31	39	39	109
2	134	52	96	282	67	81	122	270	67	86	118	271	79	100	100	279
3	104	51	88	243	64	71	95	230	65	74	96	235	71	76	91	238
4	78	38	64	180	43	54	72	169	51	52	73	176	50	52	65	167
5	67	25	54	146	32	44	62	138	43	49	61	153	45	41	58	144
6	102	55	88	245	59	80	88	227	73	83	101	257	63	60	77	200
7	91	34	66	191	45	53	58	156	44	46	61	151	53	48	53	154
8	71	31	48	150	32	41	60	133	37	40	54	131	37	35	46	118
9	101	44	83	228	47	60	66	173	58	57	63	178	54	57	58	169
10	82	45	87	214	49	66	71	186	58	60	67	185	47	52	56	155
11	103	52	98	253	57	73	85	215	65	72	80	217	60	60	74	194
12	159	67	134	360	93	100	113	306	100	98	106	304	94	99	101	294

FIG. 1.—Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of 25, 37.5, and 50 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 4, series 1)

TABLE 5.—*Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of arsenic trioxide, the chemical added after the plants were weighed on the sixth day*

SERIES 1

| Day | Loss of water in grams by control | | | | Loss of water in grams after addition of— | | | | | | | | | | | |
| | | | | | Arsenic 10 parts per million | | | | Arsenic 15 parts per million | | | | Arsenic 20 parts per million | | | |
	a	b	c	Total	a	b	c	Total	a	b	c	Total	a	b	c	Total
1	67	58	60	185	99	81	72	252	70	62	87	219	93	74	62	229
2	90	73	79	242	115	108	96	319	89	82	109	280	120	98	80	298
3	60	47	42	149	70	59	56	185	63	52	66	181	77	55	54	186
4	110	97	95	302	137	133	110	380	124	108	136	368	156	124	100	380
5	78	66	74	218	91	91	87	269	87	63	108	258	106	92	84	282
6	95	89	81	265	117	110	93	320	117	102	126	345	140	104	92	336
7	75	62	65	202	86	92	80	258	85	71	94	250	106	90	81	277
8	118	110	95	323	130	128	115	373	132	101	142	375	141	128	105	374
9	118	110	104	332	121	113	126	360	130	97	137	364	135	121	118	374
10	145	134	119	398	145	125	147	417	151	102	157	410	137	134	137	408
11	123	118	116	357	124	104	112	340	131	90	135	356	122	108	113	343
12	75	64	58	197	76	73	65	214	76	55	86	217	77	72	68	217

SERIES 2

Day	a	b	c	Total	a	b	c	Total	a	b	c	Total	a	b	c	Total
1	244	166	188	598	170	176	133	479	203	219	204	626	161	193	214	568
2	199	142	153	494	149	145	125	419	156	183	167	506	146	159	193	498
3	252	194	194	640	190	187	165	542	176	220	228	624	196	205	242	643
4	190	154	154	498	145	172	149	466	148	195	176	519	159	165	179	503
5	268	217	221	706	213	229	203	645	205	237	244	686	241	231	266	738
6	173	135	140	448	133	164	146	443	134	164	151	449	148	142	159	449
7	150	114	117	381	105	134	133	372	111	130	126	367	125	121	137	383
8	116	87	94	297	84	107	92	283	88	101	100	289	94	92	98	284
9	180	131	132	443	133	151	136	420	135	141	143	419	146	139	152	437
10	153	122	128	403	126	161	138	425	125	138	147	410	130	135	145	410
11	148	113	120	381	129	142	121	392	119	126	131	376	131	124	140	395
12	97	80	88	265	91	111	90	292	81	100	102	283	92	91	97	280

FIG. 2.—Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of 25, 37.5, and 50 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 4, series 2.)

TABLE 6.—*Daily loss of water by Earliana tomato plants growing in 6-inch pots of garden soil, with and without the addition of sodium arsenite (0.01 gm.) and potassium arsenite (0.10 gm.) the chemicals added after the plants were weighed on the sixth day*

	Loss of water in grams by—		
Day	Plant 1, untreated	Plant 2, treated with sodium arsenite	Plant 3, treated with potassium arsenite
1	115 0	142 5	129. 0
2	113.0	135. 5	111.0
3	83. 5	102.0	83 5
4	66. 0	85.0	72 5
5	121.0	156 5	150 0
6	201.0	271. 5	246 0
7	194 5	217.0	153 5
8	231. 5	296 5	157 0
9	141 0	150 5	82.5
10	245 0	287.0	152 0
11	283 5	277.5	138 5
12	165 0	188. 5	107. 0
13	201. 5	232.0	122 0
14	182 5	222 0	111.5
15	265 0	270 5	127.0
16	250 5	242. 5	125. 0
17	210. 5	216. 5	131 0
18	167. 5	178 5	96. 0
19	104. 5	113 0	59 0
20	242. 5	245. 5	139. 0
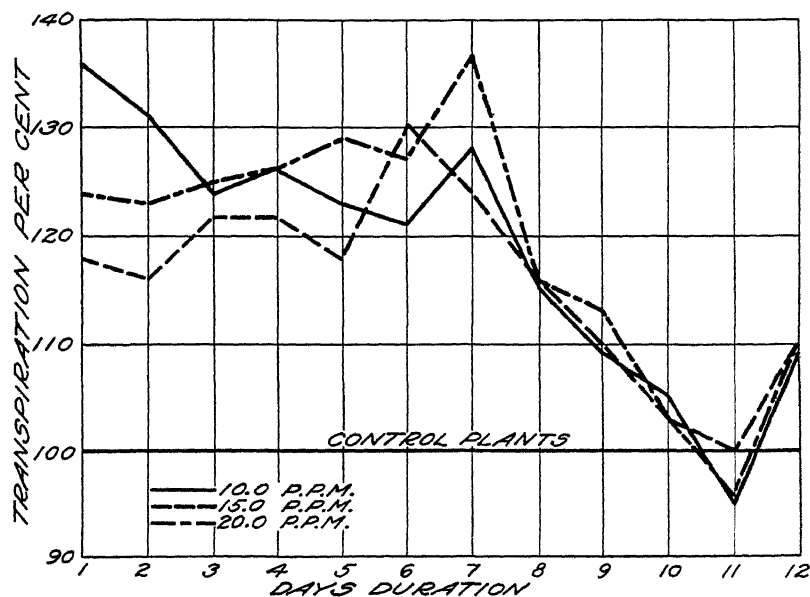
FIG 3.—Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of 10, 15, and 20 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 5, series 1.)

TABLE 7.—*Daily loss of water by Earliana tomato plants growing in 6-inch pots of garden soil. Sodium arsenite (0.05 gm.) added to each pot after the plants were weighed on the sixth day*

TREATED PLANTS

Date	Loss of water in grams by plant No						Total loss of water	Hours between weighing	Average loss (grams per hour)
	3	5	7	8	10	11			
1913									
Feb. 27	86.0	82 5	89 5	85 5	48 5	95.0	487 0	23 0	21.2
Feb. 28	78 5	85.5	98 0	99 0	50.5	103.5	515.0	25.0	20.6
Mar. 1	86.0	110.5	123.5	145.0	75 0	127 5	667.5	25 0	26.7
Mar. 2	80.0	62.0	61 5	76.5	39.0	68 0	387 0	22 0	17 6
Mar 3	127.0	132.0	141 0	155 5	89 5	149 0	794 0	24 0	33.1
Mar 4	95.5	101.5	95.0	128.0	64 0	112 0	596 0	24.0	24 8
Mar. 5	126.0	129 5	131.0	155 5	94.5	163 0	799.5	25 0	32 0
Mar 6	60 5	63.0	71 0	86 0	45 0	82 0	407.5	23 0	17 7
Mar. 7	67.0	94.5	102.0	113.5	82.0	142.0	601.0	24.0	25.0
Mar 8	90.0	113.0	118.0	108 0	82.0	117.0	628 0	24.0	26 2
Mar 9	111.0	133.0	138.0	145 0	98.0	225 0	850.0	25 0	34 0
Mar. 10	129.0	166.0	145.0	165 0	110 0	200 0	915.0	27.5	33 3
Mar. 11	37.0	47 0	37 0	43 0	28 0	47 0	239.0	19.3	12.4
Mar. 12	77.0	108 0	98 0	96 0	77 0	112.0	568.0	24 0	23.7
Mar. 13	84.0	118 0	93 0	92 0	90 0	113 0	590 0	24.0	24.6
Mar. 14	70.0	100.0	83 0	73 0	92 0	95 0	513 0	24.0	21.4
Mar. 15	113.0	169.0	130.0	114 0	159 0	178.0	863 0	25 0	36.5
Mar 16	112.0	150.0	120 0	107 0	119 0	152.0	760 0	24 5	31.0
Mar. 17	85.0	115 0	95 0	85.0	113.0	114 0	607 0	23.0	26.4
Mar. 18	66.0	111.0	82 0	82 0	131 0	140.0	612 0	21.5	25 0
Mar. 19	146.0	211.0	147.0	141.0	124.0	124 0	893 0	29.5	30.3
Mar. 20	83.0	48 0	80 0	77 0	102 0	87 0	477 0	24.5	19.5
Mar. 21	60.0	103.0	69 0	52 0	90 0	85.0	459.0	22 5	20 4
Mar 22	104.0	157.0	109.0	82.0	124 0	107.0	683 0	24.0	28.5
Mar. 23	54.0	93.0	68 0	43 0	57 0	65 0	380 0	21.0	18.1
Mar. 24	116.0	221.0	160.0	109 0	149.0	180.0	935 0	29.0	32 2
Mar. 25	84.0	122.0	87.0	57 0	83 0	90 0	523 0	24.0	21 8
Mar. 26	78.0	117 0	86.0	64 0	98.0	88 0	531 0	23 0	23 1
	2,505.5	3,263.0	2,857.5	2,779.5	2,514 0	3,361.0	17,280 5		

CONTROLS

Date	Loss of water in grams by plant No.						Total loss of water	Hours between weighing	Average loss (grams per hour)
	1	2	4	6	9	12			
1913									
Feb. 27	79.5	94.0	96.0	102 5	85.0	69.0	526.0	23.0	22.9
Feb 28	101.5	102.0	84.0	92.0	80.0	65.0	533 5	25.0	21.3
Mar. 1	105.0	100.5	118 5	112 5	109.0	81.5	627.0	25.0	25.1
Mar. 2	96.0	96.5	60.5	58 0	56 0	41.0	408.0	22.0	18.5
Mar. 3	152.0	154.0	138.5	130 0	123.0	68 0	765 5	24.0	31.9
Mar 4	124.0	119.0	92.0	90.5	81.5	57.5	564.5	24.0	23.5
Mar. 5	145.5	151.0	140 0	132.0	134.0	108.0	810.5	25.0	32.4
Mar. 6	74 0	80.0	55.5	71.0	65.0	56.0	401.5	23.0	17.5
Mar. 7	156.0	167.0	151.0	149.0	161.0	134.0	918.0	24.0	38.3
Mar. 8	176.0	204 0	157.0	181.0	160.0	117.0	995.0	24.0	41.5
Mar 9	178.0	195.0	163.0	167.0	210.0	143 0	1,056.0	25.0	42.2
Mar. 10	197.0	225.0	200 0	170 0	207.0	137.0	1,136.0	27.5	41.3
Mar. 11	77.5	81.0	73 0	47.0	56.0	43.0	377 5	19.3	19.6
Mar. 12	129.5	130.5	139.5	102.0	144 0	102.0	747.5	24.0	31.1
Mar. 13	131.0	142.0	150.0	109.0	172.0	122.0	826.0	24.0	34.4
Mar. 14	112.0	114.0	128.0	97.0	140 0	103.0	694.0	24.0	28.9
Mar. 15	176.0	219.0	208.0	169.0	239.0	183.0	1,194.0	25.0	47.8
Mar. 16	179.0	201 0	173.0	139.0	162.0	122.0	976.0	24.5	39.8
Mar. 17	131.0	143.0	159 0	105.0	152.0	117.0	807.0	23.0	35.1
Mar. 18	100.0	111.0	107.0	78.0	199.0	148.0	743.0	24 5	30.3
Mar. 19	206.0	229 0	217.0	170 0	184.0	139.0	1,145.0	29 5	38 8
Mar. 20	109.0	137.0	126 0	117.0	164.0	146.0	799.0	24.5	32.6
Mar. 21	127.0	146.0	158.0	116.0	176.0	154 0	877.0	22 5	39.0
Mar. 22	213.0	234.0	219.0	175 0	219.0	180 0	1,240.0	24.0	51.7
Mar. 23	106.0	124.0	82.0	90.0	99.0	90.0	591.0	21.0	28.1
Mar. 24	271.0	316.0	246.0	230.0	264.0	211.0	1,538.0	29.0	53 0
Mar. 25	148.0	162 0	143.0	117 0	169.0	117.0	856.0	24 0	35.7
Mar. 26	142.0	151 0	168.0	123 0	175.0	169.0	928.0	23.0	40.3
Mar. 27	273.0	309.0	249.0	214.0	246.0	209.0	1,500.0	25.0	60.0
Mar. 28	153.0	147.0	145.0	98 0	201.0	109 0	853.0	23.0	37 1
Mar. 29	116.0	85.0	99.0	57.0	73.0	43.0	473.0	24.0	19 7
Mar 30	144 0	89.0	105.0	64.0	58.0	39.0	499.0	24.0	20.8
Mar. 31	119.0	77.0	88.0	53 0	44.0	30.0	411.0	25.0	16.4
Apr. 1	81.0	63.0	72.0	42.0	41.0	28.0	327.0	23.0	14.2
Apr. 2	173 0	118.0	138.0	84.0	74.0	65.0	652 0	26.0	25.1
	5,001.5	5,216.5	4,848 5	4,051.5	4,931.5	3,746.0	27,795.5		

From a study of these tables and figures it is apparent that arsenic added as arsenic trioxide decreased the transpiration of tomato plants to a greater or less extent when the plants were grown in soil. The tables emphasize the fact that considerable variation in the quantity of water lost by the same plant occurs from day to day. These variations are due mainly to meteorological conditions.

In Figure 1 it is shown that a decided effect was produced on transpiration by the addition of arsenic trioxide in quantities of 25, 37.5, and 50 parts per million. While some variation occurred from day to day, at the end of the experiment the loss of water by the treated plants was from 80 to 85 per cent of that lost by the controls as compared to 97 per cent or more at the time the chemical was added. Figure 2, a graphic representation of a duplicate of this experiment, shows similar results.

Figures 3 and 4, where smaller quantities of arsenic trioxide were used, indicate a considerable effect on transpiration from the addition
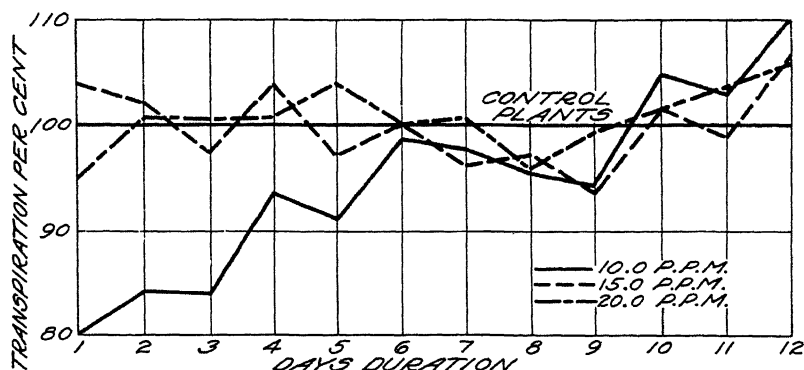
FIG. 4 —Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of 10, 15, and 20 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 5, series 2.)

of the chemical. In Figure 3 the treated plants on the sixth day averaged 126 per cent total transpiration in comparison with that of the controls, and on the eleventh day they averaged only 97 per cent, indicating that the chemical influenced transpiration.

Figure 4 shows a small decrease for three days, and then a marked tendency toward recovery. In all cases where only small quantities of arsenic trioxide were added, the plants showed a tendency to recover after a certain length of time, which in these experiments varied usually from 2 to 5 days.

The fact that the loss of water by the control plants shown in the different graphs was higher or lower than by the treated plants is not significant since there was considerable variation and all data were comparable.

TRANSPIRATION STUDIES WITH TOMATO PLANTS POTTED IN SAND

Quartz sand No. 3.5, a commercial product obtained from Ottawa, Ill., was screened through standard 20 and 40 mesh sieves, and only that part was used which passed through the 20-mesh sieve and was retained by the 40-mesh. The sand was thoroughly digested with

weak hydrochloric acid for 48 hours and then washed with distilled water until no acid reaction was noted with methyl orange as in indicator. The water-retaining capacity of the sand was about 11 per cent.

The tomato plants used in the experiments were grown in soil, and at the time of transplanting were about 5 inches high. As much of the soil as possible was removed from their roots and they were transplanted in glazed earthenware jars containing 2,500 gm. of air-dried sand. The nutrient solution (p. 3) was added to bring the moisture content to 15 per cent of the air-dried weight of the sand.

The plants were allowed to grow for about a month before the experiment was begun. During this interval an additional 125 gm. of the nutrient solution was added besides the distilled water necessary to replace the loss through transpiration.

During the experiment the jars were so sealed as to allow no loss of water except through the aerial parts of the plant. Daily an amount of distilled water equal to that lost by the plant was added. The loss was determined by weighing on scales sensitive to 0.5 gm. Arsenic trioxide in solution was added to the plants by a method similar to that described under soil cultures. The results of this experiment are shown in Table 8 and Figure 5.

TABLE 8.—*Daily loss of water by Everbearing tomato plants growing in sand, with and without the addition of arsenic trioxide, the chemical added after the plants were weighed on the sixth day*

Day	Loss of water by control				Loss of water after addition of arsenic, 10 parts per million				Loss of water after addition of arsenic, 20 parts per million			
	a	b	c	Total	a	b	c	Total	a	b	c	Total
	Gm.	Gm.	Gm.	Gm	Gm	Gm.	Gm.	Gm.	Gm	Gm.	Gm.	Gm.
1	60	35	60	155	22	32	39	93	46	47	45	138
2	90	55	98	243	30	17	60	137	75	74	75	224
3	65	40	70	175	22	32	44	98	55	51	52	158
4	101	70	112	283	38	66	76	180	89	83	91	263
5	65	49	77	191	27	43	54	124	60	57	65	182
6	102	80	110	292	48	70	80	198	97	88	109	294
7	63	52	69	184	25	30	30	85	25	33	33	91
8	71	67	90	228	19	24	26	69	21	24	20	65
9	82	81	107	270	13	27	33	73	18	22	22	62
10	106	105	142	353	23	39	46	108	20	29	27	76
11	54	45	59	158	16	20	26	62	14	19	19	52
12	43	35	57	135	16	23	28	67	16	25	23	64

From these it is apparent that arsenic decreases the transpiration of tomato plants when they are grown in sand. Table 8 shows that considerable variation in the loss of water occurs from day to day, a fact which is mainly due to meteorological conditions. The injury is apparent in a shorter period of time in sand than in soil, when equal quantities of arsenic trioxide are added and transpiration is used as a criterion. Compare Figure 3 with Figure 5. After the fourth day there is a marked tendency toward recovery.
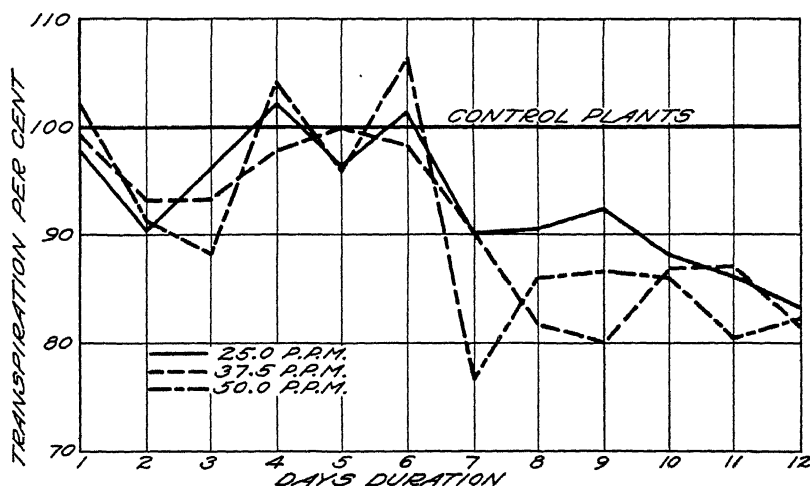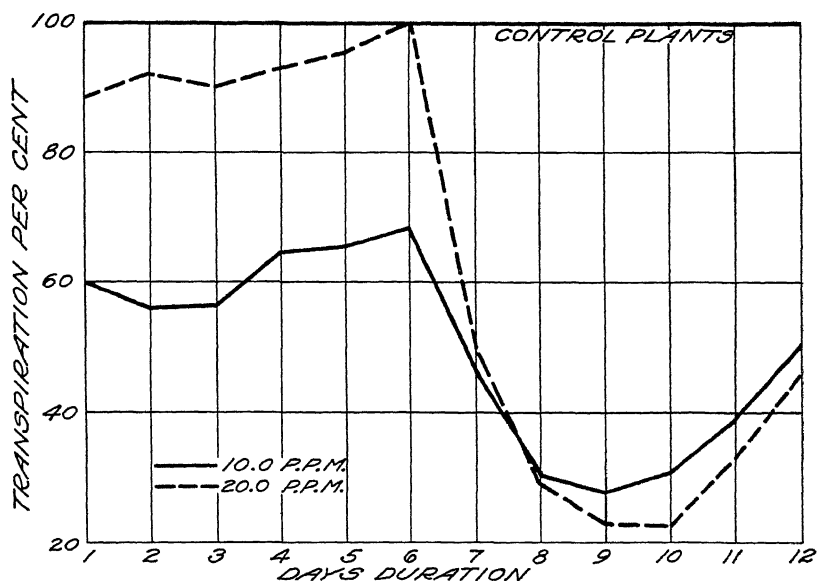
Fig 5 —Daily loss of water by Everbearing tomato plants growing in sand, with and without the addition of 10 and 20 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants   (Data from Table 8)

EFFECT ON GROWTH OF POTTED PLANTS CAUSED BY ADDITION OF SOLUBLE ARSENICAL COMPOUNDS TO THE SOIL

The plants in this group of experiments were grown in 6-inch pots full of garden soil.   Table 9 gives the variety of plant and its condition at the time of treatment, and Table 10 shows the effect produced by the chemicals on the various plants.   Potassium or sodium arsenite proved to be highly toxic to practically all the plants.   The other compounds were only slightly toxic to the cereals.   In the same family or group there was noted a wide variation in tolerance of the chemical, as, for example, arsenic acid and potassium arsenate in the quantities added proved fatal to radish and caused only slight injury to turnip.   Sodium arsenate killed the oat plants, but apparently caused no injury to barley or wheat.

TABLE 9.—*Condition of plants prior to treatment with soluble arsenicals as shown in Table 10*

Plant	Variety	Height in inches	Condition
Barley	New Zealand	10–14	Stems slender, normal color.
Bean A	Prolific German Wax	15–25	Vigorous growth, normal color.
Bean B	White Navy	15–25	Vigorous growth, normal color
Cabbage	Large Premium	6–12	Fair condition, light green in color.
Clover	Mammoth Red	5	Leaves normal in size and color
Corn	White Flint	21–27	Stalks slender, normal color.
Cucumber	Improved Long Green	8–12	Vigorous growth, normal color.
Lettuce	Hanson	6	Leaves 4–5 inches wide, normal color.
Oats	Swedish Select White Wonder	10–18	Stems weak but erect, normal color
Pea	Canadian Field	7–22	Somewhat spindling growth, normal color.
Radish	White Strasburg	8–14	6–8 leaves, stems slender, normal color.
Squash	Hubbard	8–12	Vigorous growth, normal color.
Timothy		12–20	Spindling growth of stems, light green color
Tomato A	Early Michigan	8–10	Vigorous growth, normal color.
Tomato B	Perfection	8–10	Vigorous growth, normal color
Turnip		6–12	Roots small to 2 inches in diameter, normal color.
Wheat	Glyndon 650	14–20	Stems somewhat weak, normal color.

TABLE 10.—*Effect on potted plants caused by the addition to the soil of 0.5 gm. of soluble arsenicals*

Plant used	Effect on plant caused by addition to soil of—					
	Arsenic acid	Ammonium arsenate	Potassium arsenate	Sodium arsenate	Potassium arsenite	Sodium arsenite
Bailey	No injury	No injury	No injury	No injury	Very bad	Dead
Bean A	Dead	Very bad	Dead	Dead	Dead	Do
Bean B		Dead	....do	....do	....do	Do
Cabbage						Do.
Clover				Dead		Do.
Corn	No injury	No injury	Moderate	No injury	Very bad to dead	Do.
Cucumber	Dead	Dead				Do
Lettuce	....do		No injury	No injury	Dead	Do
Oats	No injury	No injury	....do	Dead	Bad	Do
Pea						Do
Radish	Dead	No injury	Dead	No injury	Dead	Do
Squash				....do		Do
Timothy						Do
Tomato A	Dead	Dead	Moderate	No injury	Dead	Do
Tomato B	Bad	....do	No injury	....do	....do	Do
Turnip	No injury	No injury	Slight	....do	No injury	Bad
Wheat	....do	...do	No injury	....do	Dead	Dead

EFFECT ON GROWTH OF POTTED PLANTS CAUSED BY ADDITION OF INSOLUBLE ARSENICAL COMPOUNDS TO THE SOIL

The plants in these experiments were grown in 6-inch pots full of garden soil. Table 11 shows the effect on growth produced by adding 2.5 gm. of the arsenical, which was sprinkled as evenly as possible over the surface of each pot. The control plants made a satisfactory growth, and remained in a good healthy condition throughout the experiment.

TABLE 11.—*Effect on the growth of various plants in 6-inch pots caused by the addition to the soil of 2.5 gm. of arsenic compounds*

Arsenic compound used	Effect of arsenical on—					
	Bailey	Carrot	Oats	Sugar beet	Tomato A	Wheat
Arsenic (metal)	Very injurious	Dead				
Arsenic trioxide	No injury	Slightly stunted			Slightly stunted.	
Calcium arsenate	....do	Moderately stunted			...do	
Copper arsenate		Badly stunted	No injury		No injury	
Ferrous arsenate		....do			...do	
Lead arsenate		Slightly stunted	No injury		... do	
Mercury(ic) arsenate		....do				No injury.
Zinc arsenate		Badly stunted		Dead	Dead	Do.
Calcium arsenite				...do		Dead.
Copper arsenite				No injury	Slightly stunted.	Dead
Iron and ammonia arsenite.		Moderate stunted		No injury		Dead.
Zinc arsenite		....do		...do		
Arsenic sulphide, red.				...do		
Lead arsenite				No injury	Injurious.	

The so-called "insoluble" arsenical compounds are arranged below in the order of their toxicity as indicated by the injury which they caused to bean and tomato. Those listed first caused severe injury; those preceded by an asterisk caused none.

BEAN

Amonium arsenate	Lead arsenite	Lead arsenate
Zinc arsenate	Copper arsenite	Mercuric arsenate
Arsenic metal	Zinc arsenite	Ferric arsenite and ammonium citrate
Calcium arsenite	Arsenic trioxide	
Mercurous arsenite	Calcium arsenate	*Arsenic disulphide, red
Arsenic sulphide, yellow	Ferrous arsenate	*Copper arsenate

TOMATO

Arsenic metal	Arsenic sulphide, yellow	*Ferrous arsenate
Calcium arsenite	Zinc arsenite	*Lead arsenite
Ammonium arsenate	Arsenic trioxide	*Ferric arsenite and ammonium citrate
Mercurous arsenite	Mercuric arsenate	
Copper arsenite	*Calcium arsenate	*Arsenic disulphide, red
Zinc arsenate	*Copper arsenate	*Lead arsenate

These results indicate that plants show an individuality in their reaction to chemical compounds, which may vary greatly with different species.

EFFECT ON GROWTH OF CROPS CAUSED BY INCORPORATION OF ARSENICAL COMPOUNDS IN THE SOIL

The ground selected for the work reported in this section was a uniform strip of medium clay loam slightly sloping to the west. The ground was prepared by the ordinary method used for making a good seed bed. It was then staked out into plots 3 feet square with 2-foot alleys running north and south and east and west. Figure 6 shows the arrangement of the plots, the cropping system, and the arsenical compounds used. This system remained uniform throughout the experiment unless otherwise noted. Each year the plots except the alfalfa, clover, and timothy were spaded, thoroughly worked, and planted. The original planting was as follows: Wheat, timothy, alfalfa, and clover in 5 rows running north and south, and 6 inches apart. Sugar beets and field peas in 3 rows running north and south, and 12 inches apart. The first row in all cases was 6 inches from the margin of the plot. Potatoes, cabbage, and tomatoes were set 4 plants to a plot, equidistant and 10 inches from the margin.[3] Cucumbers were planted about 2 inches apart in a circular hill 16 inches in diameter.

The plots were watered by a revolving sprinkler to prevent any washing of the surface soil. The chemicals used and the quantities added at each treatment were as follows (each plot received an equivalent amount of arsenic, approximately 11 gm., as determined by the results of analysis made by members of the chemistry department of the Montana station):

Chemical	Grams per treatment	Chemical	Grams per treatment
Arsenic trioxide	14. 58	Lead arsenate ertho	63. 10
Arsenic trisulphide	18. 03	Lead arsenite	30. 50
Calcium arsenite	20. 82	Sodium arsenite	18. 33
Copper aceto-arsenite	24. 55	Sodium arsenate	43. 07
Lead arsenate pyro	50. 00	Zinc arsenite	20. 10

[3] The cabbage and tomato plants were transplanted from 4-inch pots when they were about 5 inches tall

TABLE 12.—*Growth of plants in plots of soil treated with different arsenicals*

CABBAGE

Year	Plot treated with—											
	Arsenic trioxide	Arsenic trisulphide	Calcium arsenite	Copper aceto-arsenite	Lead arsenate acid	Lead arsenate ortho	Lead arsenite	Sodium arsenate	Sodium arsenite	Zinc arsenite	Control 1	Control 2
1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	Fair	Moderate	Fair	Poor	Moderate	Poor	Fair	Fair	Moderate	Poor	do	Do.
1913 [b]	do	Poor	None	do	do	Good	Poor	None	Poor	None	do	Do.
1914 [c]	Moderate	Fair	Poor	do	Good	Moderate	None	Poor	None	Fair	do	Moderate.
1915 [f]	Good	do	do	do	do	Good	Good	None	Moderate	do	do	Good.
1916	Fair	Poor	do	Moderate	Moderate	Moderate	Fair	Fair	Fair	Poor	do	Do.
1917 [a]	Moderate	Fair	do	Fair	Good	Good	Moderate	Moderate	Moderate	Fair	do	Do.
1918	None	None	Moderate	Moderate	Fair	Moderate	Good	Moderate	Good	Poor	do	Moderate.
1919	Poor	Moderate	Fair	Fair	Moderate	do	Fair	Moderate	do	Fair	do	Good.
1920	do	Fair	do	do	do	do	Good	Moderate	Fair	do	Moderate	Do.
1921	do	Fair	do	do	Good	do	Fair	Fair	Fair	do	Moderate	Do.
1922	Good	Good	Good	Good	Good	Good	do	Good	Good	Good	Good	Do.

CUCUMBER

Year	Arsenic trioxide	Arsenic trisulphide	Calcium arsenite	Copper aceto-arsenite	Lead arsenate acid	Lead arsenate ortho	Lead arsenite	Sodium arsenate	Sodium arsenite	Zinc arsenite	Control 1	Control 2
1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	Moderate	Fair	None	Poor	Moderate	Moderate	Poor	Poor	Fair	None	do	Do.
1913 [b]	None	None	do	None	None	None	None	None	None	do	do	Do.
1914	do	do	do	do	do	do	do	do	do	do	Moderate	Do.
1915 [a]	do	do	do	do	Poor	do	do	Poor	do	do	Good	Do.
1916	do	Poor	do	Poor	do	do	do	do	do	Poor	do	Do.
1917	Poor	Poor	Poor	None	do	Poor	do	do	Poor	Fair	do	Do.
1918	Poor	Fair	do	Fair	do	None	Poor	do	do	Poor	do	Do.
1919	do	Poor	do	Poor	do	do	do	Fair	do	Fair	do	Do.
1920	do	do	do	do	Fair	do	do	do	do	do	do	Do.
1921	do	Fair	do	do	Poor	Poor	do	do	do	Fair	do	
1922 [d]												

FIELD PEA

Year	Arsenic trioxide	Arsenic trisulphide	Calcium arsenite	Copper aceto-arsenite	Lead arsenate acid	Lead arsenate ortho	Lead arsenite	Sodium arsenate	Sodium arsenite	Zinc arsenite	Control 1	Control 2
1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	do	Moderate	Moderate	Moderate	do	do	Moderate	Moderate	Moderate	Poor	do	Do.
1913	do	Poor	None	Fair	do	do	Poor	Poor	Fair	None	do	Do.
1914	do	do	do	Poor	Moderate	Fair	do	do	Poor	do	do	

POTATO

Year									
1915 b	Moderate	Fair	Fair	Fair	Moderate	Fair	do	Fair	Do
1916	Poor	None	None	None	Poor	None	None	None	Do.
1917	do	Poor	Poor	Poor	Fair	Poor	do	Poor	Do.
1918	do	Moderate	Fair	Moderate	Fair	Fair	Fair	Fair	Do.
1919	Good	Fair	Poor	do	do	do	Moderate	Good	Do.
1920	Moderate	Poor	Fau	Fair	Moderate	Poor	Moderate	Poor	Do.
1921	Fair	Moderate	Poor	Poor	Poor	do	Good	Fair	Do.
1922	Good	Good	Good	None	Good	Good	Fair	Moderate	Do
							Good	Good	

Year									
1911 a	Good	Poor	Fair	Good	Moderate	Fair	Moderate	Fair	Good
1912	do	Good	Good	do	Good	Good	Good	Good	Do.
1913 b	do	do	do	do	do	Moderate	Moderate	do	Do.
1914	do	Fair	Poor	Moderate	Moderate	Fair	Poor	Poor	Fair.
1915 b	Moderate	Poor	Fair	Good	Good	do	do	do	Moderate.
1916	Good	Fair	do	do	do	Moderate	do	do	Do.
1917 a	do	do	do	Moderate	Moderate	Fair	Moderate	Moderate	Good.
1918	Fair	Good	Good	Good	Good	Fau	Fair	do	Do.
1919	do	Fair	do	do	Good	do	do	do	Do.
1920	Moderate	Poor	Fair	do	Moderate	Good	Moderate	Fair	Do.
1921	Fair	Moderate	Moderate	do	Moderate	do	Moderate	do	Moderate.
1922	Poor	Fair	do	do	do	Good	do	do	Good.
	Good	Good	Good	Good	do	do	Good		

TOMATO

Year									
1911 a	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	do	do	do	do	do	do	do	do	Do.
1913 b	Moderate	Fair	Moderate	Moderate	Moderate	Moderate	Moderate	Poor	Do.
1914	do	Fau	do	do	do	Fair	Fair	do	Do.
1915 b	do	do	do	do	do	do	Moderate	do	Do.
1916	Good	Moderate	Moderate	Moderate	Good	do	Poor	None	Do
1917 a	Moderate	Poor	None	None	Moderate	do	Poor	do	Do.
1918	Poor	None	Poor	Poor	Good	Poor	do	do	Do.
1919	do	Poor	Moderate	Good	Good	Moderate	do	do	Do.
1920	Moderate	Moderate	do	do	do	Fair	Moderate	Moderate	Do.
1921 e	Good	Fair	do	do	do	Moderate	Good	Fair	Fair.
1922	Moderate								

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a Chemical treatment given that year.
b Treatment before planting and after harvest.
c Growth was generally very poor in 1911 on account of injury caused by worms.
d Plots not planted.
e No difference observed.

TABLE 12—*Growth of plants in plots of soil treated with different arsenicals*—Continued

SUGAR BEET

Year		Plot treated with—										
	Arsenic trioxide	Arsenic trisulphide	Calcium arsenite	Copper aceto-arsenite	Lead arsenate acid	Lead arsenate ortho	Lead arsenite	Sodium arsenate	Sodium arsenite	Zinc arsenite	Control 1	Control 2
1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	do	Poor	Moderate	Moderate	Moderate	Moderate	Fair	Moderate	do	Fair	do	Do.
1913 [b]	Poor	Moderate	Fair	do	Fair	Fair	do	Poor	Fair	Very poor	do	Do.
1914	Good	Good	Very poor	Poor	Good	Moderate	Moderate	do	do	None	do	Do.
1915 [b]	do	Moderate	Fair	Moderate	do	Good	do	do	Moderate	do	do	Do.
1916	Fair	Poor	None	Poor	Fair	Moderate	Poor	None	None	do	do	Do.
1917 [a]	Poor	Very poor	do	None	Moderate	Fair	do	Poor	Poor	Good	do	Do.
1918	Moderate	Fair	Good	Moderate	Good	Good	Moderate	do	Fair	do	Moderate	Moderate.
1919	Fair	Good	Fair	Fair	Fair	Fair	do	Poor	Poor	Moderate	Good	Fair.
1920	Moderate	Good	Poor	do	do	do	Fair	Moderate	Good	do	do	Good.
1921	Fair	Good	Moderate	do	Good	Moderate	Moderate	Fair	Fair	do	do	Do.
1922	Moderate	Fair	do	do	Good	Moderate	Good	Moderate	Moderate	do		

WHEAT

Year		Plot treated with—										
	Arsenic trioxide	Arsenic trisulphide	Calcium arsenite	Copper aceto-arsenite	Lead arsenate acid	Lead arsenate ortho	Lead arsenite	Sodium arsenate	Sodium arsenite	Zinc arsenite	Control 1	Control 2
1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good
1912	do	do	Moderate	Moderate	do	do	do	Fair	do	Fair	do	Do.
1913 [b]	do	do	Poor	Fair	Moderate	do	Poor	None	Poor	None	do	Do.
1914	do	Poor	do	Poor	do	Moderate	do	do	None	do	do	Do.
1915 [b]	Moderate	Fair	Fair	Fair	Moderate	Fair	Fair	Poor	Poor	Fair	do	Do.
1916	do	do	Poor	Poor	Fair	do	Poor	None	None	Poor	do	Do.
1917 [a]	do	do	None	None	do	Moderate	do	Poor	Poor	None	do	Do.
1918	None	None	Poor	Fair	Moderate	do	None	do	Fair	do	do	Do.
1919	do	Poor	Fair	Fair	do	do	Fair	Fair	None	Poor	do	Do.
1920	Poor	None	Poor	Fair	do	Good	Poor	Moderate	Fair	None	do	Do.
1921	do	do	do	Fair	do	do	Fair	do	Fair	do	Moderate	Do.
1922	do	do	Fair	do	do	do	Poor	Good	Poor	do		

[a] Chemical treatment given that year.
[b] Treatment before planting and after harvest.

FIG. 6.—Diagram showing arrangement of plots, cropping system, and arsenical used in experiments made to determine the effect on the growth of crops caused by the incorporation of arsenical compounds in the soil

During the first year only the chemical was added by sprinkling it evenly over the surface of the soil while the crops were growing. All the other treatments were given in the fall after the crops were harvested, or in the spring before planting and incorporated in the soil.

The alfalfa, clover, and timothy plots proved very unsatisfactory owing to winter-killing and the intrusion of other grasses and weeds. These plots were abandoned and no detailed notes were taken on them.

Tables 12 and 13 indicate the time of treatment and the yearly rating of the season's growth of the different crops, namely, cabbage, cucumber, field peas, potatoes, tomato, sugar beet, and wheat, and the toxicity of the various arsenicals in relation to the crop.

TABLE 13.—*Toxicity of arsenicals to different crops* [a]

[Ranking from 1 (poor growth) to 10 (approximately normal growth)]

Arsenical	Toxicity of arsenical to—					
	Cabbage	Peas	Potato	Tomato	Sugar beet	Wheat
Arsenic trioxide	5	9	9	8	6	8
Arsenic trisulphide	6	5	3	4	9	7
Calcium arsenite	3	2	4	2	2	3
Copper aceto-arsenite	2	4	5	5	8	4
Lead arsenate acid	10	10	10	9	10	10
Lead arsenate ortho	9	8	8	10	7	9
Lead arsenite	8	1	7	6	3	6
Sodium arsenate	4	7	6	3	1	2
Sodium arsenite	7	3	2	7	4	5
Zinc arsenite	1	6	1	1	5	1

[a] All arsenicals injured the growth of cucumbers to such an extent that ranking was impossible.

From a critical examination of the data contained in Table 12 it appears that some chemicals become inactive very much sooner than others and that the amount of injury caused by the chemicals varies from year to year with different crops. In all the experiments the final addition of arsenic was made in 1917, and the plots were continually cropped until 1922 in order to determine the rapidity with which the soil would again produce normal growth. At no time did lead arsenate cause much injury, whereas zinc arsenite caused severe injury throughout the entire time. The degree of injury attributable to the other chemicals ranged between these extremes, as is shown by the data in Table 12.

SUMMARY

The results of transpiration studies with oats in water cultures show conclusively that arsenic added as arsenic trioxide decreases transpiration even when added at the rate of one part per million. The characteristics of treated plants are narrower leaf blades and a lighter color. There is apparent individuality in the reaction of plants to this arsenical.

Decreased transpiration resulted when arsenic trioxide was added at the rate of 10 parts per million or more to soil in which tomato plants were growing, and this decrease was more apparent in direct

proportion to the amount of arsenic added until serious injury or death resulted.

Similar results were obtained when sodium arsenite or potassium arsenite was substituted for arsenic trioxide.

When sand was used instead of soil, the other environmental conditions remaining the same, the injury was apparent in a shorter time. Considerable variation occurred from day to day, mainly due to meteorological conditions.

The addition of small quantities of soluble arsenical compounds to potted plants caused serious injury to most of the plants under test. As a rule the cereals were hardier than the other crops. Turnip was also fairly resistant. The arsenites were decidedly more toxic than the arsenates.

Some of the so-called "insoluble" arsenical compounds proved very toxic to plants when 2.5 gm. of the chemical was sprinkled evenly over the surface of the pot.

The toxicity of arsenical chemicals to plants varied. Some species showed a high, and to others a low, degree of tolerance to the same arsenical.

The incorporation of arsenical compounds in the soil is a dangerous practice, and may cause considerable injury as the concentration of arsenic increases. Arsenical compounds differ in their reaction in the soil, some becoming inert in a much shorter period than others. Plants also differ in their ability to withstand arsenic, as is illustrated by the fact that some crops remain approximately normal when arsenic in some form is present, while other crops in the same environment are killed. Beans and cucumbers are very susceptible to arsenic, but the cereals and grasses are much more resistant.

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STUDIES OF COMMERCIAL SAUERKRAUT WITH SPECIAL REFERENCE TO CHANGES IN THE BACTERIAL FLORA DURING FERMENTATION AT LOW TEMPERATURES[1]

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INTRODUCTION

The fermentation of cabbage is a spontaneous one, and the manufacturer of sauerkraut must take the undesirable microorganisms along with the desirable ones. Because of this chance inoculation, there is a wide variation in the quality of sauerkraut. A large variety of organisms is introduced with the green plant tissue when this is placed in the vats. Many of the forms on plants can not survive under the conditions in the vat, while others find the conditions very favorable and increase to enormous numbers.

Changes in oxygen tension, hydrogen-ion concentration, and fermentable carbohydrate bring about conditions which for a few days are favorable for certain kinds of bacteria, but within a few days more these conditions may change and become unsuitable for that particular kind of microorganism. A new flora better adapted to the new conditions may come to the front and persist for a shorter or longer period of time. Although the general character of the fermentation is similar in all vats, no two are exactly the same.

The extensive research which has been made on silage shows clearly that the souring of plant tissue is the result of the combined action of many types of microorganisms. Although there are decided differences between the formation of silage and of sauerkraut, the main outlines of the two processes are the same and many of the same bacteria are found in both fermentations.

REVIEW OF LITERATURE

In the older literature on sauerkraut undue emphasis probably was placed on the activities of one type of microorganism. Conrad (4),[2] the first investigator in this field, isolated a culture which he regarded as the principal agent in the fermentation and to which he gave the name *Bacterium brassicae acidae*. It was a motile, Gram-negative rod which, among other products, produced hydrogen and methane. Most of the bacteria which have since been obtained from sauerkraut are nonmotile, Gram-positive, and produce neither hydrogen nor methane. *Bacterium brassicae acidae* is clearly not the usual kind of bacteria which occurs in sauerkraut.

In 1903 and 1905 Wehmer (23, 24) published two papers on several aspects of sauerkraut fermentation. He concluded that gas formation was due to alcohol-forming yeasts and that acid production was caused by an organism which he tentatively named *Bacterium brassicae*. This organism was a short rod which at times appeared to be almost

a coccus and did not form any gas. Wehmer gives such a meager description of his organism that it is practically impossible to identify it with any culture which has since been isolated from sauerkraut. In a recent publication LeFevre (*12*) has concluded that *Bact. cucumeris fermentati*, isolated by Henneberg (*9*), and *Lactobacillus pentoaceticus*, isolated by Fred, Peterson, and Davenport (*5*), are identical with Wehmer's *Bact. brassicae*. While Wehmer says nothing of the products formed by *Bact. brassicae* other than that it formed acid, he repeatedly emphasizes the fact that *it did not form gas*. Gas production is an outstanding characteristic of *L. pentoaceticus*. Approximately 25 per cent of the glucose and other sugars is converted into carbon dioxide. Because of this and other differences it seems certain that *Bact. brassicae* and *L. pentoaceticus* are different microorganisms.

About the same time that the papers of Henneberg and Wehmer appeared, Butjagin (*3*) published a paper in which he described the successive changes in the flora of sauerkraut. He isolated and gave a good description of a short round-ended rod which in many respects resembled *Bacterium guntheri* of Gunther and Thierfelder. Aderhold (*1*) also isolated a similar organism from pickle and sauerkraut fermentations. Butjagin's bacterium differed from *Bact. guntheri* in that it did not grow well in milk and coagulated milk only after 21 to 23 days. It was Gram-positive, nonmotile, and produced no gas. In respect to the last two characteristics it was like Wehmer's *Bact. brassicae*. Wehmer makes no mention of the reaction of his organism to Gram's stain, but it is probable that it was positive. Probably both organisms are members of a large group of bacteria occurring in silage, sauerkraut, and pickles, which are strong acid producers but do not form gas. A detailed examination of the reaction of the non-gas-producing sauerkraut bacteria toward various sugars and other organic compounds shows marked differences among the various members with respect to their ability to ferment these compounds.

Shortly before Wehmer's long paper on sauerkraut appeared, Perekalin (*14*) published a short note about an acid-tolerant bacterium which he had isolated from sauerkraut. This organism was a short, nonmotile rod, negative to Gram's stain, and did not produce spores nor coagulate milk.

In 1909 Gruber (*8*) isolated an acid-producing culture which he believed to be characteristic of sauerkraut. This organism resembled Conrad's *Bacterium brassicae acidae*. It was motile, produced gas, and in other respects resembled the colon type of bacteria. He named his organism *Pseudomonas brassicae acidae*.

Round (*17*) published a short note in 1916 regarding the large numbers of bacteria in commercial suaerkraut, and concluded that bacteria alone are responsible for the fermentation. An organism which he isolated was studied by Fred, Peterson, and Davenport (*5*) in connection with the fermentation of xylose. These investigators isolated a large number of similar organisms from silage, sauerkraut, soil, manure, and cereals. They belong to the so-called "mannitol-forming" bacteria, and have received considerable attention in connection with the production of wine and butyl alcohol. In wine they produce a sour, bitter taste and in the butyl-alcohol fermentation they inhibit the growth of the organism which produces butyl alcohol.

In 1916 Henneberg (*10*) published a series of papers in which he discussed the manufacture of sauerkraut from many different angles. He divided the bacterial flora of sauerkraut into 9 or 10 groups. In the early days of the fermentation, bacteria of the colon-aerogenes type may be present, but Henneberg does not believe that they serve any useful purpose and should be excluded if possible. Oval-shaped forms, similar to *Streptococcus lactis*, are also frequently found in the early stages of the fermentation, but these gradually die off as a considerable degree of acid is attained. Henneberg attributes the odor and taste of sauerkraut chiefly to the activities of alcohol, acetic-acid-producing lactics such as *Bacterium brassicae fermentatae*. Other aroma-producing bacteria are the Pediococcus forms, of which there are many varieties. Particularly abundant in sauerkraut are the short rod forms to which group the incompletely described *Bact. brassicae* of Wehmer belongs. In old, strongly acid sauerkraut *Bact. cucumeris fermentati* and a gas-producing variety of this form are common. Other bacteria, which are less clearly defined, are chain types, flocculating types, and slime-producing types.

The functions of yeasts in the production of sauerkraut is still an unsettled question. Wehmer attached great importance to their presence, while Butjagin suggested that they might be more or less harmless, nonessential organisms. The esterlike flavors have been attributed to the compounds formed between the alcohols produced by the yeast and the acids produced by the bacteria. Peterson, Fred, and their associates (*16, 21*) have shown that the mannitol-forming bacteria convert 25 per cent of the glucose and other sugars into ethyl alcohol. It is therefore not necessary to have yeasts in sauerkraut in order to have alcohol present.

Fred and Peterson (*6*) also showed that the so-called "pink" sauerkraut is due to yeasts. Under certain conditions the sauerkraut may become infected with large numbers of wild yeasts which produce a dirty brown color and an offensive odor and taste. On the basis of existing data no final conclusion can be drawn as to the rôle of yeasts in sauerkraut fermentation.

In 1925 Brunkow, Peterson, and Fred (*2*) published the results of a systematic study of the number and kinds of bacteria which occur in experimental sauerkraut at successive times during the fermentation period. It was desired to extend this study to commercial sauerkraut and to make a somewhat more thorough investigation of the different forms, especially as regards the occurrence of the mannitol-forming bacteria. The present paper deals with the results of this investigation.

METHODS

SOURCE OF SAMPLES

Most of the samples of sauerkraut juice were secured from a commercial sauerkraut factory, which will be designated as factory A. The samples were taken from four large vats, each vat containing about 40 tons of sauerkraut. Spigots were placed on the side near the bottom of the vats. This made it possible to draw off the juice as needed without disturbing the sauerkraut. Before a sample was taken to be analyzed some of the juice was allowed to flow through the spigot. At the beginning of the fermentation samples

were drawn at frequent intervals, but in the last part of the fermentation period samples were taken about every 30 days.

Samples of sauerkraut juice which had been in the vats from 41 days to 1 year were obtained from another factory, B.

TEMPERATURE OF VATS, ACIDITY OF SAUERKRAUT, AND NUMBER OF BACTERIA

To obtain the temperatures at which fermentation took place, a pointed metal tube was forced into the sauerkraut in such a way that the lower end was approximately at the center of the vat. Inside the tube was a thermometer, the lower part of which was inserted through a cork into a slender test tube filled with water. By this arrangement no apparent change in temperature took place for several minutes after the thermometer was withdrawn from the tube. The temperature was also taken of the sample as it was drawn from the spigot. The two temperature readings varied only a few degrees.

A 10 c. c. sample of the sauerkraut juice was diluted with an equal volume of water and, after boiling for a few seconds to expel the CO_2, was titrated with 0.1 N NaOH to the end point of phenolphthalein.

Counts of the number of bacteria were made by plating the sauerkraut juice on glucose-yeast-water agar which had been adjusted to P_H 6.8. The plates were incubated at 28° C. and counted after 48 hours and again after 96 hours of incubation. After counting, 10 colonies were picked from one of the plates from each vat. As a rule the picking was made from the plate on which from 30 to 50 colonies were growing. The plate was sectioned off until there were about 10 colonies to the section. The section which appeared to have the most representative growth was selected and *all the colonies picked*. In this way 80 cultures were obtained from each of the four vats. Forty cultures were picked from the four samples of 41-day sauerkraut obtained from factory B and 10 cultures from the 1-year-old sauerkraut; in all, 370 cultures were picked.

DISCUSSION OF DATA

The temperature, acidity, and number of bacteria in the vats at various times during the fermentation are shown by the curves in Figures 1 to 4. The outstanding fact regarding these sauerkrauts is the low temperature at which fermentation took place. At the time the vats were filled the temperature was almost freezing, and at no time during the fermentation did it rise above 10.5° C. Most sauerkraut manufacturers would regard these as unusually low temperatures, but it must be noted that Wehmer considers 6° to 8° C. as the proper temperature at which to make sauerkraut. That the low temperature reduced the number of bacteria is evident from the curves in Figures 1 to 4. The highest number of bacteria in a normal fermentation is usually found from about the fifth to the seventh day. In the four vats studied the maximum was not reached until between the fifth and the twentieth day. Vat 3, which had the lowest average temperature, had an unusually low count throughout the entire fermentation, and the maximum number was not obtained until the twentieth day.

After the maximum was reached the count for all the vats rapidly dropped to a small number and continued at this low level for several weeks. After 90 days the temperature of the vats rose slightly, and
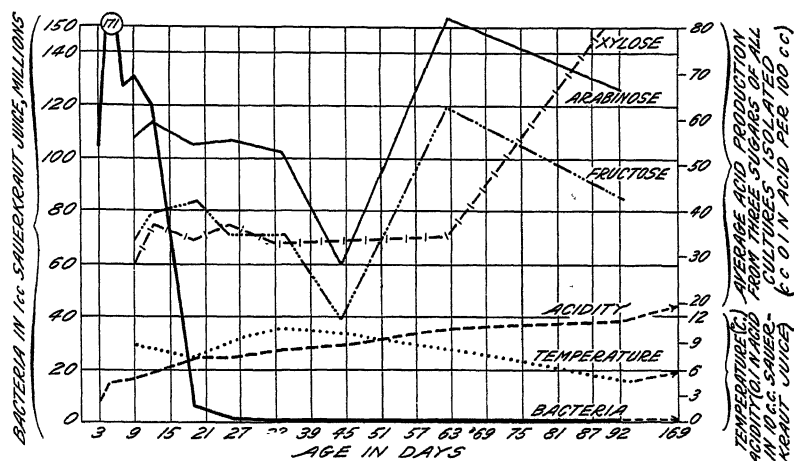
FIG 1.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut, also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 1)

this rise was accompanied by a small increase in the number of bacteria.

In the first days of the fermentation numerous peptolytic catalase-positive bacteria were present, but these soon disappeared. Coccus
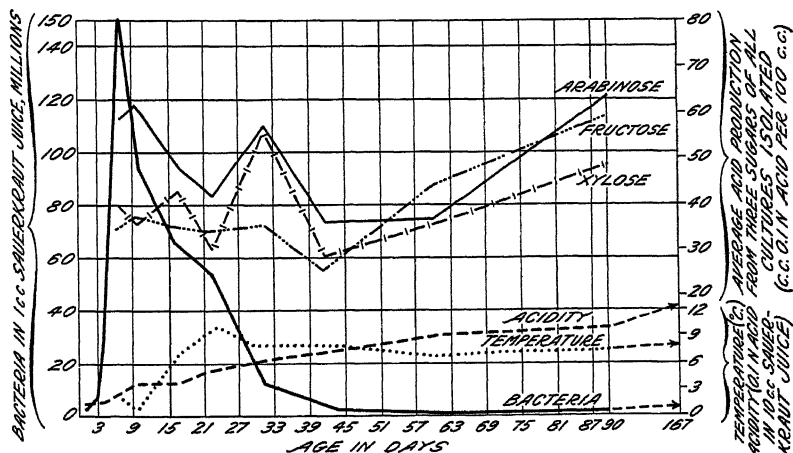
FIG. 2.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 2)

forms predominated in the early stages of the fermentation; short rods were present all the time, but were more abundant during the latter part, and long rods appeared only toward the end of the fermentation. All four vats showed a slow increase in acid, but none

reached a particularly high amount.   At the end of 90 days about 1 per cent of acid, calculated as lactic, was found.   Samples of the sauerkraut juice were then analyzed for reducing sugars, and the
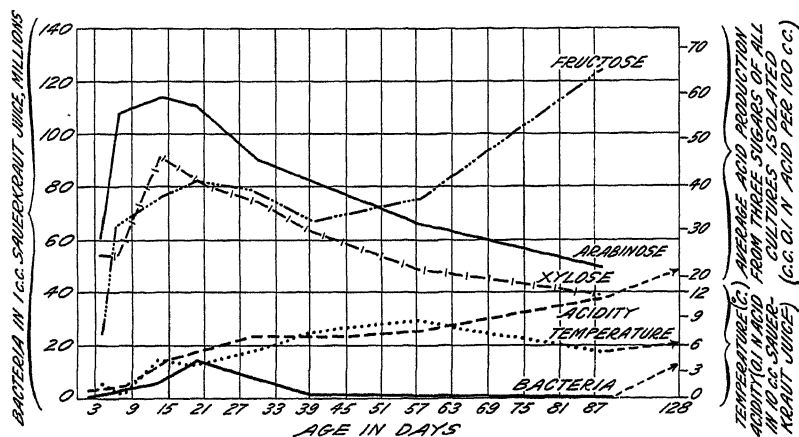
Fig 3 —Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut, also the acidity produced from certain sugars by sauerkraut bacteria   (Vat 3)

surprising fact was revealed that there was practically none left. Apparently complete fermentation had taken place at the low temperatures of the vats, but only a small part of the sugar had been converted into acids.   A complete analysis of the sauerkraut juice
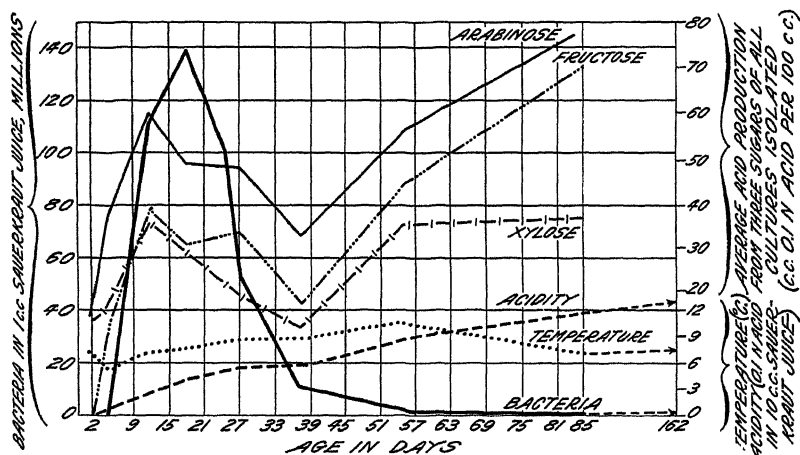
Fic. 4.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria.   (Vat 5)

was therefore made to determine, if possible, what other fermentation products had been formed.   The methods used have been described in previous publications (*18, 19, 5, 15*).

TABLE 1.—*Fermentation products contained in sauerkraut juice from different vats (factory A)*

No of vat	Temper-ature [a]	Age of sauer-kraut	Sugar	Titrat-able acid as lactic	Volatile acid as acetic	Nonvola-tile acid as lactic	Alcohol as ethyl
	° C.	Days	Per cent	Per cent	Per cent	Per cent	Per cent
Vat 1	8.0	92	0.12	1.026	0.270	0.663	0.432
Vat 2	6 9	90	.10	873	.266	.575	.653
Vat 3	5.3	87	.12	1.017	.276	.703	.920
Vat 5	7 7	85	10	.972	.334	678	.680

[a] Average for entire fermentation period, from 128 to 169 days.

FERMENTATION PRODUCTS

The percentage of volatile acid, nonvolatile acid, alcohol, and unfermented sugar contained in the sauerkrauts are given in Table 1. The percentage of acetic acid is normal but the amount of lactic acid is low. In a normal fermentation there is from three to five times as much lactic acid as acetic acid, but in the sauerkrauts studied the ratio is only from 2 to 2.5. The alcohol content ranged from 0.43 to 0.92 per cent, with an average of 0.67 per cent. The average for nine commercial sauerkrauts was found by Fred and Peterson (7) to be 0.47 per cent. The analytical data indicate that the low temperatures of these vats favored the development of alcohol-producing bacteria. It is possible that, if mannitol could have been determined on these samples, an unusually high percentage would have been found. No satisfactory determination could be obtained because of the interference of salt. When the vats were opened the sauerkraut was graded and was rated fair. From this limited evidence it would seem that low temperatures do not give the best quality of sauerkraut. More data must be obtained, however, before a final conclusion on this point can be drawn.

KINDS OF BACTERIA IN THE SAUERKRAUTS

A rough classification of the several hundred bacteria picked from the plates was made by inoculating 10 c. c. of 1 per cent yeast-water solutions of fructose, arabinose, and xylose with these cultures and determining the acidity formed after seven days of incubation. The titrations for the 10 cultures picked at each time of sampling were averaged for each sugar and the results plotted in the form of curves as shown in Figures 1 to 4.

While there are many irregularities in the curves, the acid production from these sugars seems to follow the bacterial curve during the first half of the fermentation. When the number of bacteria decreased, the acid-producing ability of the bacteria also decreased. The decline in acid production was not so great, however, as in numbers of bacteria, and after six weeks a general increase in acid production took place. A high acid-producing type of bacteria came to the front. These were probably the long rods which are usually abundant in old sauerkraut.

The cultures were also inoculated into litmus milk, but less than 50 per cent produced any reduction or curd in this medium and only a few digested milk. Those which did produce proteolytic changes in litmus milk were cultures which had been obtained in the early

stages of the fermentation. These bacteria were undoubtedly plant forms which did not find the conditions of the vat suitable for their existence.

The cultures were also tested for catalase with hydrogen peroxide, but more than 80 per cent were found negative. The great majority of bacteria in sauerkraut are catalase-negative, produce little or no change in milk, but ferment arabinose, xylose, and fructose readily with the production of considerable quantities of acid. The large number of pentose fermenters corroborates the findings reported in a previous paper (2) in which it was stated that the xylose fermenters practically equalled the number that attacked glucose.

FERMENTATION CHARACTERISTICS OF THE SELECTED CULTURES

On the basis of the above five tests 90 of the 320 cultures were selected for further study. In making this selection an effort was made to include representatives of all the various types encountered. Tables 2 to 5 give the representative types found in the four commercial vats at factory A. Table 6 contains the data for the selected cultures obtained from factory B. Numbers 60–2, 60–6, and 60–11 were selected from the year-old sauerkraut and all others in this table came from the 41-day sauerkraut. A few of the organisms in Table 6 are outstanding because of the great acidity developed in the sugars. Organisms in Tables 2 to 5, selected from factory A, where a low temperature was maintained, do not show such a marked ability to produce acid. Most of the cultures slowly produce acid in litmus milk and about half of them bring about some other change such as curdling, reduction, or granule formation. The mannitol formers produce less acidity and softer curds than the nonmannitol bacteria. Reduction of litmus is also more prominent with the latter group.

TABLE 2.—*Fermentation characteristics of the selected cultures from vat 1 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c*	*C. c.*
40–1	No change	a +	72	54	78. 9	50. 0
40–3	___do___	+	62	22	75. 8	47. 2
40–6	___do___	+	76	50	73. 1	48. 4
41–2	___do___	+	72	50	75. 9	71. 6
41–10	___do___	+	78	48	75. 9	46. 8
43–8	___do___	+	78	50	84. 8	52. 0
44–1	___do___	+	88	62	75. 9	53. 6
45–3	___do___	+	69	50	86. 0	50. 0
46–2	___do___	+	51	115	96. 0	58. 0
46–8	Reduced	+	98	114	90. 0	51. 2
46–10	___do___	+	102	109	100. 0	55. 2
47–9	No change	+	106	120	94. 0	50. 4
48–1	___do___	+	36	112	80 0	49. 0
48–3	___do___	+	134	107	82. 0	50. 0
48–6	Reduced	−	79	28	96. 0	97. 0

a Plus sign indicates presence of gas; minus sign, absence of gas.

TABLE 3.—*Fermentation characteristics of the selected cultures from vat 2 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	C. c.	C. c.	C. c.
50-2	No change	−	82	52	81.8	52.0
50-5	....do....	+	72	66	85.0	50.4
51-9	Soft curd....	+	86	50	82.0	53.2
53-4	No change	+	24	68	81.8	52.8
53-5	....do....	+	70	58	58.4	51.6
53-8	....do....	+	22	78	80.5	52.0
54-1	....do....	−	18	26	54.0	54.0
54-5	....do....	+	74	56	82.4	56.4
54-10	Granules	−	74	24	62.4	73.0
55-4	No change	+	90	52	84.0	52.0
55-6	....do....	+	32	76	82.0	51.6
55-9	....do....	+	80	80	88.0	54.4
56-2	Reduced	+	76	59	102.0	52.4
56-7	No change	−	15	16	86.0	32.8
57-2	Reduced	−	19	21	98.0	69.2
57-3	....do....	−	120	28	90.0	60.8
57-7	....do....	+	115	114	86.0	45.2
57-8	No change	+	67	60	88.0	47.2
58-3	....do....	−	22	18	96.0	82.0
58-6	....do....	+	120	128	102.0	78.0
58-8	Reduced	−	106	32	104.0	104.0

TABLE 4.—*Fermentation characteristics of the selected cultures from vat 3 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	C. c.	C. c.	C. c.
21-1	No change	+	64	54	76.0	50.4
21-2	Reduced, curd	+	36	22	54.0	4.8
22-3	No change	+	82	26	77.4	48.4
22-5	....do....	+	94	54	90.5	47.6
22-8	....do....	+	72	46	62.8	93.2
22-10	Peptonized	+	34	40	53.1	8.0
24-1	No change	+	80	74	74.4	54.4
25-6	....do....	+	86	60	80.5	55.6
25-7	Reduced, curd	−	76	70	65.6	71.6
26-4	Reduced	+	62	46	84.0	62.4
26-8	No change	+	64	59	57.2	90.0
27-1	Reduced	+	63	44	78.0	49.2
27-8	....do....	+	78	62	84.0	45.2
28-5	No change	−	59	19	64.0	60.4
28-7	Reduced	+	66	104	80.0	58.4
28-8	No change	−	19	25	88.0	67.6
29-1	Reduced	−	66	38	94.0	99.0
29-4	No change	+	127	115	104.0	73.0
29-6	....do....	−	21	19	104.0	97.0
29-7	....do....	−	28	22	82.0	99.0

TABLE 5.—*Fermentation characteristics of the selected cultures from vat 5 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c c of culture			
			Arabinose	Xylose	Glucose	Fructose
			C c.	*C. c.*	*C c.*	*C c.*
30-2	Reduced, curd	+	42	30	39. 4	3 6
30-7	Reduced	+	27	48	50 6	11. 6
31-1	No change	+	94	24	74 5	48 0
31-2	Reduced, curd	−	10	28	55. 0	12. 0
31-8	----do	+	40	38	40. 8	8 4
32-2	----do	−	70	70	73 6	57. 6
32-6	No change	+	88	50	73. 1	55. 2
33-1	----do	+	72	50	50 0	70. 1
33-2	----do	+	72	44	71. 6	50 8
34-2	Reduced	+	80	42	90 0	51. 2
34-4	----do	+	53	39	88 0	51 2
34-7	----do	+	66	76	84. 0	47. 6
35-1	No change	−	65	17	74 0	22 8
35-7	Reduced	+	78	52	90 0	48 8
35-10	----do	+	61	42	86. 0	50. 8
36-2	Reduced, curd	−	81	35	100 0	95 6
36-4	Reduced	+	108	133	100 0	51. 6
36-6	Reduced, curd	−	23	34	108. 0	88. 0
37-2	Reduced	−	109	28	104 0	94. 0
37-3	----do	+	70	48	92. 0	91. 0
37-6		+	102	125	88. 0	75 0

TABLE 6.—*Fermentation characteristics of the selected cultures from factory B*

Culture No.	Litmus milk after 30 days	Gas from glucose	0 1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
60-2	Reduced	+	120	116	86	106
60-6	----do	+	84	105	98	54
60-11	Reduced, curd	−	27	25	106	100
62-3	----do	−	20	23	90	96
62-6	No change	+	120	109	126	76
62-10	Reduced, curd	−	73	39	78	101
63-5	Reduced	+	119	109	96	71
64-2	Reduced, curd	−	69	30	100	91
64-7	Reduced	+	118	22	84	118
64-8	----do	−	20	24	88	154
64-9	Reduced, curd	+	114	106	92	87
65-5	----do	−	66	34	98	201
65-9	Reduced	−	19	22	90	152

GAS FORMATION

The cultures were inoculated into 10 c. c. of 1 per cent glucose-yeast water, sealed with sterile melted vaseline and allowed to ferment at 28° C. They were observed from day to day for gas formation. If the vaseline plug was pushed up they were recorded as gas-positive. If the vaseline remained at the surface of the liquid and there was no apparent leakage of gas, they were called negative. At the end of seven days the vaseline plug was removed and the culture titrated for acidity with 0.1 N. NaOH. If acid had developed it was evident that the organisms had grown and the negative results were not due to a failure to grow.

Of the 90 cultures, 63 formed gas and 27 did not. The proportion of gas-forming organisms was higher in the vats from factory A than

from factory B. In the first factory there were almost three times as many gas formers as nongas formers, while in the second factory the numbers were about equal. Low temperatures appear to favor the development of gas-producing bacteria. As will be shown later, gas and mannitol formation coincide in most cases. Certain cultures, however, produced no gas but formed a small quantity of mannitol.

REACTION TO GRAM'S STAIN

In most cases the gas-producing organisms were also positive to Gram's stain. Most of the organisms were short rods, but some of those picked from the young sauerkraut were cocci. In the later stages of the fermentation a considerable number of long rods appeared.

RESELECTED AND PURIFIED CULTURES

On the basis of the tests just described the 90 cultures were gone over and 18 were chosen as representative of all the types found in the larger group. These were replated and new colonies picked. The purified cultures were inoculated into 10 c. c. of 1 per cent yeast-water solution of melezitose, raffinose, and salicin, and after seven days' incubation titrated for acid. Their ability to form mannitol from fructose was also determined by fermenting 75 c. c. of 2 per cent fructose-yeast water containing an excess of $CaCO_3$. After seven days the fermented medium was carefully decanted from the excess $CaCO_3$ and evaporated to 5 to 10 c. c. on the steam bath and then allowed to go to dryness at room temperature. If the culture is a good mannitol former, crystals of mannitol will appear throughout the residue and frequently extend up the sides of the beaker. The test is usually very definite, but if doubt existed the fermentation was repeated. The cultures in which no crystals appeared the second time were acidified with H_2SO_4 and extracted four times with ether to remove lactic acid, which tends to hide the mannitol crystals. The residue from the ether extraction was then extracted six times with hot 80 per cent alcohol and the extract concentrated to a small volume on the steam bath and then evaporated spontaneously at room temperature. If no mannitol crystals appeared the cultures were classed as nonmannitol-forming. The results of this test and the other characteristics of the 18 cultures are summarized in Table 7. This list includes cultures which ferment both pentoses, some of which ferment arabinose but not xylose, and some which ferment neither. Only 3 cultures were obtained which fermented xylose but not arabinose. Such a microorganism has been reported in a previous publication (*21*), but the arabinose-negative, xylose-positive type is much less common than the reverse combination.

Only four cultures were found to ferment melezitose, and all of these were mannitol-formers. The converse is not true, however; several cultures formed mannitol but did not ferment melezitose.

If 25 c. c. of 0.1 N acid be taken as indicating fermentation, more than half of the 18 cultures attack raffinose and salicin. According to Orla-Jensen (*13*), the ability to ferment salicin is rare among the lactic-acid bacteria of green vegetable matter.

TABLE 7.—*Fermentation characteristics of selected cultures*

Culture No	Gas	Litmus milk	Mannitol formation	0 1 N acid in 100 c. c. of culture						
				Arabinose	Xylose	Glucose	Fructose	Melezitose	Raffinose	Salicin
				C. c.	C. c.	C. c.	C. c.	C. c.	C. c.	C. c.
29-7	−	−	−	28	21	82	99	16	16	56
36-6	−	+	+	23	34	108	88	75	89	74
54-10	−	−	−	74	24	63	62	24	33	25
25-7	−	+	−	76	70	67	72	17	19	33
32-2	+	+	+	70	70	73	58	17	58	55
31-2	−	+	−	10	28	55	12	8	18	29
64-2	−	+	+	69	30	100	91	67	85	80
57-8	+	−	+++	67	60	88	47	17	25	18
58-8	−	+	+	106	32	104	104	84	63	88
22-10	+	+	−	34	40	53	8	23	40	27
46-2	+	−	+++	51	115	96	58	19	18	20
51-9	+	+	+	86	50	80	53	15	43	80
22-8	+	−	++	72	46	63	93	17	48	57
55-9	+	+	++	80	80	88	54	20	42	48
48-3	+	−	+++	134	107	82	50	28	22	23
36-4	+	+	+++	108	133	100	51	19	20	21
64-9	+	+	++	114	106	92	87	19	62	23
26-8	+	−	++	64	59	57	90	17	38	52

A large majority of the organisms are mannitol formers but vary in the quantity which they produce. Those forming a small quantity are indicated with one plus sign in the table, while those forming a large quantity are indicated with two or three plus signs. Three of the mannitol formers, Nos. 36–6, 64–2, and 58–8, were conspicuous because of the large deposit of calcium salts which separated out with the mannitol crystals. They also failed to form gas from either glucose or fructose. The tests for gas and mannitol formation were repeated twice to make sure there was no error. The quantity of mannitol was small but unmistakable. Apparently the formation of mannitol is a property which varies from traces to 50 to 60 per cent of the fructose consumed. In 1921 Kayser (*11*) reported a similar case of mannitol formation by lactic-acid bacteria which did not form any gas.

Several of the cultures are noteworthy in that they produce no turbidity in the media. Flocceules appear on the sides of the tube or settle to the bottom, leaving the medium perfectly clear. Cultures 25–7 and 64–9 were conspicuous in this respect. Considerable importance has been attached to the property of floccule formation in a recent classification of lactic-acid bacteria by Van Steenberge (*20*)

QUANTITATIVE DETERMINATION OF THE FERMENTATION PRODUCTS FROM ARABINOSE, FRUCTOSE, AND GLUCOSE

Six of the mannitol formers were selected for quantitative work. Differences in the fermentation of the sugars formed the basis of the selection. From the following summary it can be seen at a glance what the differences are. The minus sign signifies that there was little or no fermentation; the plus sign indicates good fermentation, and the double plus very good fermentation.

Culture No.	Arabinose	Xylose	Glucose	Fructose	Melezitose	Raffinose	Salicin
36-6	−	−	+	+	+	+	+
64-2	+	−	+	+	+	±	+
57-8	+	+	+	+	−	−	−
36-4	++	++	+	+	−	−	−
55-9	+	+	+	+	−	+	+
64-9	+	+	+	+	−	±	−

The cultures were inoculated into 300 c. c. of sugar-yeast-water media containing about 2 per cent of the sugar. The fermentation flask was equipped with a trap and absorption bottle for collecting the CO_2. Sterile bromo-cresol purple was added to the flask, and as fermentation proceeded the acid which formed was neutralized with sterilized 1 N NaOH. When acid production ceased the culture was acidified with H_2SO_4 and aspirated for 30 minutes to remove and absorb any traces of CO_2 in the flask. The KOH solution was made to a definite volume and analyzed for CO_2 by the Van Slyke (22) apparatus. The fermented medium was made to 400 c. c. and aliquots analyzed for unfermented sugar, alcohol, volatile acid, nonvolatile acid, and mannitol by methods already described (18, 19, 5, 15). The data are given in Table 8.

TABLE 8.—*Fermentation products of arabinose, glucose, and fructose*

[Calculated for 100 c c of culture]

Culture No.	Age	Sugar		Acids		Alcohol as ethyl	Carbon dioxide	Manni- tol	Total products
		Kind	Amount fermented	Volatile as acetic	Nonvola- tile as lactic				
	Days		Gm.	Gm.	Gm.	Gm.	Gm.	Gm	Gm.
57-8	7	Arabinose	1 823	0 841	0 937	0.012	0.040	----------	1.830
36-4	7	do	1.829	.806	.983	.005	.036	----------	1.830
55-9	12	do	1.138	.427	.674	.006	.026	----------	1.133
58-8	11	do	1.938	.347	1.412	.008	.047	----------	1.814
64-9	8	do	1 864	.379	1.485	005	.041	----------	1.910
64-2	8	do	1.938	.505	1 319	008	.062	----------	1.894
57-8	36	Glucose	1 225	.085	438	.248	270	----------	1 041
64-9	34	do	1 700	133	.620	.297	.365	----------	1.415
36-4	15	do	.747	032	.281	.141	.116	----------	.590
55-9	15	do	1.497	047	.658	.319	.314	----------	1.338
64-2	34	do	1.110	.051	846	.008	.042	----------	.947
36-6	36	do	1.940	.066	1.728	019	.046	----------	1.859
57-8	12	Fructose	1.550	.288	.377	.055	.223	0. 516	1.459
64-9	14	do	1.615	257	.677	.129	.344	.232	1.639
36-4	12	do	1 580	.299	.580	.082	.246	.173	1.380
55-9	8	do	1.068	.234	.438	.051	.159	.103	.985
36-6	17	do	1.550	.044	1.429	.029	.066	?	1.568
64-2	14	do	1.615	.052	1.558	.013	.048	?	1.671

FERMENTATION OF ARABINOSE

This sugar was fermented very vigorously by all the cultures with the exception of No. 36-6 which does not attack this pentose. The principal products formed were volatile and nonvolatile acids. Only traces of CO_2 and alcohol were formed.

There was great variation in the quantity of products formed. The volatile acid comprised from 19 to 47 per cent and the nonvolatile acid from 50 to 78 per cent. On the basis of arabinose fermented, the lactic acid amounts to more than 70 per cent of the sugar. If more than 60 per cent of the sugar is converted into lactic acid, it is necessary to assume that parts of two molecules of arabinose are drawn upon to produce the extra 10 or more per cent of lactic acid. Only one 3-carbon compound can be obtained per molecule of pentose unless the remaining 2-carbon portions are first combined and then broken down to yield a 3-carbon compound such as lactic acid. A synthesis as well as a cleavage must therefore be brought about by the

bacteria. Cultures 58–8, 64–2, and 64–9 are all outstanding because of their ability to convert more than 60 per cent of the arabinose into lactic acid.

FERMENTATION OF GLUCOSE

The six cultures fall into two groups. Nos. 36–6 and 64–2 are straight lactics, while the other four form considerable quantities of alcohol and carbon dioxide with a small amount of volatile acid. These four cultures break up the glucose approximately as follows: $C_6H_{12}O_6 = C_3H_6O_3 + C_2H_5OH + CO_2$.

The actual figures show a slight departure from the theoretical, but in most cases this is less than 5 per cent.

FERMENTATION OF FRUCTOSE

Fructose was rapidly and almost completely destroyed. Large quantities of lactic acid were formed by Nos. 36–6 and 64–2, but no mannitol could be found. Whether mannitol is formed under the somewhat acid conditions of the fermentation or whether its presence could not be detected because of interfering substances is not known. Mannitol was formed in the presence of $CaCO_3$, but the amount was too small to determine quantitatively. The remaining four cultures were very similar in the products formed. The volatile acids varied from 16 to 24 per cent.

Mannitol, carbon dioxide, and acetic acid production run parallel to one another and inversely to the production of lactic acid. The cultures which produced much mannitol also produced large quantities of CO_2. Appreciable quantities of ethyl alcohol were formed only by such cultures as produced considerable quantities of mannitol.

FORM OF LACTIC ACID PRODUCED

The form of lactic acid is readily identified by the water of crystallization of its zinc salt. Table 9 gives the data for these salts. Most of the lactic acid produced by these organisms was inactive. Culture 55–9 differs from the other cultures in producing an excess of one form of lactic acid. The water of crystallization is intermediate between that for inactive and active zinc lactate. To determine which enantiomorph was in excess, the zinc salts were examined in a polariscope. The first crop from arabinose and fructose was tested in each case and both were found dextrorotatory. Since the rotation of the zinc salt is opposite to that of the free acid, evidently culture 55–9 produces more *l*- than *d*-lactic acid.

TABLE 9.—*Forms of lactic acid produced by various cultures*

Culture No.	Sugar	Zinc lactate			Extracted acid recovered as zinc salt
		Crop No.	Weight	Water of crystallization a	
			Gms.	*Per cent*	*Per cent*
64-2	Glucose	1	0.8173	18.1	65
64-9	____do____	1	.5711	18.1	60
36-6	____do____	1	2 1144	18.1	81
57-8	____do____	1	.4116	18 0	59
64-2	Fructose	1	2 0902	18 1	81
64-9	____do____	1	.6636	17 7 ⎫	51
		2	.1284	17.5 ⎭	
36-4	____do____	1	.5875	18.2 ⎫	52
		2	0666	18 1 ⎭	
36-6	____do____	1	2.2923	18 2	78
55-9	____do____	1	.1022	15 4 ⎫	40
		2	.1913	13 8 ⎭	
57-8	____do____	1	.4626	17 8 ⎫	51
		2	0436	17.9 ⎭	
64-2	Arabinose	1	1 5252	18 4 ⎫	61
		2	.1281	17.8 ⎭	
64-9	____do____	1	1 7636	18.2	96
36-4	____do____	1	1.1992	18.5 ⎫	88
		2	.1631	17.2 ⎭	
55-9	____do____	1	.7467	14 3	68
57-8	____do____	1	1.0091	18.5 ⎫	92
		2	.3527	18.5 ⎭	

a Theory for H_2O in $Zn(C_3H_5O_3)_2+3H_2O=18.2$; theory for H_2O in $Zn(C_3H_2O_3)_2+2H_5O=12.9$.

SUMMARY

The acidity, temperature, and number of bacteria in four vats of commercial sauerkraut were determined at different intervals for a period of more than five months. There was a slow but regular increase in acid which, calculated as lactic, was equivalent to about 1 per cent after three months, and about 1.3 per cent after five months. The temperature in all of these vats was unusually low. At no time did it rise above 10.5° C., and this fact was undoubtedly responsible for the slow development of acid. There was a rapid increase in the number of bacteria from the second to the twentieth day, after which there was a big drop in the number of microorganisms.

Pure cultures were picked from each plating until a total of 370 were obtained. Most of these organisms fermented arabinose, xylose, and fructose but produced little change in litmus milk. Practically all were catalase-negative.

The fermentation characteristics of representative cultures of the 370 were studied further. A few of these were coccus forms but most of them were rods. The large majority were Gram-positive and catalase-negative. About two-thirds formed gas from glucose and approximately the same number formed mannitol from fructose. Several new strains of mannitol-forming bacteria were found.

Six of the mannitol-forming cultures were used for the quantitative determination of their fermentation products. Lactic acid was the principal product, and varied from 36 per cent in the case of glucose to 79 per cent in the case of arabinose. Such a large percentage of lactic acid can not be obtained by direct cleavage of the pentose molecule, but must involve synthesis of portions from two or more molecules. In most cases approximately equal quantities of *d*- and

l-lactic acid were produced. One culture, No. 55–9, produced an excess of the *l* form.

Besides lactic acid, acetic acid, and ethyl alcohol, carbon dioxide, and mannitol were produced in quantities which varied with the sugar and the culture used.

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EFFECT OF PHOSPHORUS ON THE COMPOSITION OF THE TOMATO PLANT [1]

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INTRODUCTION

A large amount of research has been undertaken in the attempt to determine the effects of phosphorus on plant growth. Experiments with tomatoes have been confined almost wholly to field plots. There is obvious need of repeating some of this experimentation in sand cultures, where it is possible to control the environment more closely and partially escape the complexity of factors found under field-plot conditions. On account of the importance of chemical analyses of plant tissues in the study of the effect of different environments on plant metabolism, an attempt was made to apply this method of investigation in determining the effects of variations in the supply of phosphorus on the growth and fruitfulness of the tomato (*Lycopersicon esculentum* Mill.). This paper reports some of the results of such an attempt.

METHODS

Tomato seed, of the Bonny Best variety, was used in all experiments and was obtained as commercial package stock from a seed company in Madison, Wis. The seeds were sown in greenhouse soil in flats, and the seedlings were transplanted to soil in 3-inch pots several days after the appearance of the first true leaf. Two weeks before they were transplanted to sand, the plants were shifted to 4-inch pots. The plants used for experimental purposes were about 15 inches high, and flower buds were making their appearance.

When the plants were transplanted to sand in the first experiment, the soil was washed from the roots, and the secondary roots were removed with a pair of shears. These secondary roots were cut off because of the large amount of time it would require to free them from all soil and organic matter, and also because the decay of these roots would add organic matter to the sand. The plants send out adventitious roots from the stems for their subsequent development. In the later experiments, by properly hardening the plants it was possible to cut the stems off at the surface of the ground and by removing some of the lower leaves, to transplant them directly to

the sand as cuttings (fig. 1). The sand used was furnished by a
sand company in Ottawa, Ill. This sand contained 99.8 per cent
silica. The plants were grown in 2-gallon glazed jars having a
drainage hole at the bottom on one side. Two plants were grown
in each pot to which phosphorus was added, and either three or four
plants were grown in each pot to which phosphorus was not added.
The sand was kept moist enough to encourage plant growth by
applying city tap water as frequently as necessary.

The leaves were kept dry by watering the plants carefully and
by using wire screens to keep the leaves above the sand (9).[3] All
fallen leaves were saved. The following modification of Knop's
nutrient solution was used.

FIG. 1.—Plants in an ideal condition for transplanting by cuttings. Secondary roots have developed on the stem above the ground

Solution 1—Plus-phosphorus solution:
  (A) Magnesium sulphate, 2 per
        cent.
      Potassium nitrate, 2 per
        cent.
      Monobasic potassium phos-
        phate, 2 per cent.
  (B) Calcium nitrate, 4 per cent.
      Calcium sulphate, 2 per cent.
      Calcium chloride, 3 per cent.

Solution 2—No-phosphorus solution:
  (A) Magnesium sulphate, 2 per
        cent.
      Potassium nitrate, 2 per
        cent.
      Potassium chloride, 2 per
        cent.
  (B) Calcium nitrate, 4 per cent.
      Calcium sulphate, 2 per cent.
      Calcium chloride, 3 per cent.

The above stock nutrient solutions were diluted for use before
application by mixing 1 part of A, 1 part of B, and 10 parts of tap
water. Once or twice a week the jars were treated with this diluted
solution, to which was added 4 c. c. per liter of a 1 per cent solution
of ferric citrate. Some plants were given solution 2, which contained
no phosphorus; while other plants were given solution 1, which
contained an ample supply of phosphorus. In experiment H the
low-phosphorus plants were given an insufficient supply of phosphorus
by using a solution composed of 9 parts of solution 2 and 1 part of
solution 1. All flowers were hand-pollinated.

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[3] Reference is made by number (italic) to "Literature cited," p. 126.

PRESERVATION OF SAMPLES AND PREPARATION FOR ANALYSIS

The plants were cut for samples about 1 p. m. on a sunny day. The leaves and stems were preserved immediately, usually before 2 p. m. The remaining tissue was preserved as soon as possible. The samples, after being cut into half-inch pieces, were dried for 30 minutes at 98° C. in a steam oven, and still further dried at 60° to 65°, being well ventilated until thoroughly dried. The drying process usually required 24 hours. Samples of roots were obtained by overturning the pots, removing the roots, and washing them moderately with water. No attempt was made to wash out all the sand before drying them. After the roots were dry they were ground gently in a large mortar with a pestle until they were free from sand. The material was placed on a large sheet of paper and the roots and sand were separated by blowing the roots away from the sand.

The dried material was ground with an electric-driven pestle mill until the material was of sufficient fineness to pass through a sieve with 60 meshes to the linear inch.

One-gram samples were dried at 100° C. for 48 hours before being weighed, and after another 20 hours of drying they were reweighed. In all cases a vacuum was used during the last hour of the drying.

One gram of plant material was placed in a porcelain crucible, and 15 c. c. of saturated alcoholic solution of magnesium nitrate was added. This was then dried at 65° C. A large part of the carbon was expelled by igniting the dried material with a fuse of quantitative filter paper before the material was placed in an electric furnace in which it was heated until all the carbon had disappeared. The analysis was completed by the official volumetric method of the Association of Official Agricultural Chemists. The phosphomolybdate precipitate was filtered on a Gooch crucible, a paper-pulp mat being used.

One gram of dry powder was used to determine the total nitrogen by the official Kjeldahl-Gunning-Arnold method.

QUANTITATIVE SEPARATION OF NITROGENOUS CONSTITUENTS

Approximately 200 grams of fresh plant tissue were weighed out and chopped into fine pieces in a wooden bowl. Three 15-gram samples were weighed out in evaporating dishes for moisture determination, and three 5-gram samples were similarly weighed out for total nitrogen determination. One hundred grams of the tissue was crushed in a large mortar with a pestle. About 15 c. c. of ether was added to plasmolyze the cells, and angular quartz sand (previously washed and dried) was added to facilitate the grinding. The tissue was ground to a fine pulp, usually with the addition of more ether, and water if necessary.

The product of the above procedure was transferred to a piece of long cloth on a large funnel and was extracted by applying 2 liters of distilled water in about 30 washings. After the extraction, the solution was filtered through paper pulp and filter paper on a Büchner funnel, and made up to a definite volume of 2 liters.

The nitrogen constituents were determined on the following fractions by the total-nitrogen method previously mentioned: 150 c. c. of the above solution was used for the determination of water-soluble nitrogen; 300 c. c. aliquots were heated to boiling, 2 c. c. of 10 per

cent acetic acid was added, and the mixture was boiled for two minutes, then filtered hot into a fluted filter and washed with 100 c. c. of hot water, and the total nitrogen determined both on the coagulum and the filtrate; 600 c. c. of this filtrate was made up to a liter in volume and preserved by the addition of chloroform and toluene until the next day, when the nitrates were determined by the Strowd method (14).

Between 3 and 5 grams of dry material was placed in a 300 c. c. Erlenmeyer flask and was extracted with 90 per cent alcohol under a reflux condenser on an electric hot plate. The alcohol was kept boiling for two hours. This material was filtered and washed with an equal volume of boiling 90 per cent alcohol. The extract was evaporated on an electric hot plate at about 65° C., water being added until the extract was free from alcohol. The solution was cleared with neutral lead acetate, nearly deleaded with sodium sulfate, and the deleading was completed with sodium carbonate, phenolphthalein being used as an indicator. By the use of phenolphthalein a very distinct termination of the deleading can be easily seen. The solutions were at all times filtered through dry filter paper into dry vessels. The solution was made up to volume and the reducing power determined.

One-half of the solution prepared for reducing sugars was boiled for 45 minutes with $2\frac{1}{2}$ per cent hydrochloric acid for total hydrolysis of sugars. The solution was neutralized, made up to volume, and the reducing power determined.

The dry material from which the sugar had been extracted, as indicated above, was placed in a beaker, and 50 c. c. of water was added. The dry material was well mixed with the water and then allowed to stand at room temperature for 12 hours or overnight. After the material had been filtered and washed with cold water, the filtrate was boiled for two and one-half hours on a sand bath with $2\frac{1}{2}$ per cent hydrochloric acid. The solution was neutralized, clarified, and deleaded, and the reducing power was determined on the filtrate after it was made up to volume. It is assumed that this fraction contains the dextrins and soluble starch.

After the water had been extracted the residue was placed in the previously used beaker with 50 c. c. of water and brought to boiling and kept boiling for two minutes under a low flame to gelatinize the starch. The material was cooled to 38° C. by using a water bath, and 10 c. c. of fresh saliva (not over one hour old) was added. The hydrolysis of the starch was allowed to continue until testing with iodine solution showed that the starch had disappeared. The material was again brought to a boil in order to test for completeness of hydrolysis and to facilitate filtration. After it had been filtered, the solution was boiled for two and one-half hours on a sand bath with $2\frac{1}{2}$ per cent hydrochloric acid. As clarification was usually not necessary, the solution was neutralized, made up to volume, and the reducing power determined.

The residue was washed into an Erlenmeyer flask with $2\frac{1}{2}$ per cent hydrochloric acid and boiled for two and one-half hours on a sand bath. The solution was filtered, neutralized, clarified, and made up to volume, and the reducing power determined. This fraction is called hemicellulose in all tables.

The reducing power of all carbohydrate solutions was determined by obtaining the reduced copper under the Munson and Walker conditions. The amount of reduced copper was determined by the Shaffer and Hartmann titration method (*12*). The tables for the Munson and Walker conditions were used to find the copper equivalent in glucose. All carbohydrates are expressed in percentages of glucose.

Fresh material was cut into pieces one-half inch in length and placed in a fixative of 4 c. c. of 4 per cent formaldehyde and 96 c. c. of 60 per cent alcohol. The material was cut in paraffin on a sliding microtome and stained with safranine.

RESULTS

Experiments A, B. C, and E were preliminary and are not reported here.

EXPERIMENT D

The seed for this experiment was planted July 9, 1923; the seedlings were transplanted to 3-inch pots July 21, 1923; and to 4-inch pots August 3, 1923. The plants were placed in sand under the nutrient treatment September 3, 1923. The no-phosphorus plants, 101 in number, were sampled for analysis October 25, 1923, and the ample-phosphorus plants, 50 in number, October 26, 1923 (fig. 2). Thus the groups had been under nutrient treatment 52 and 53 days, respectively.

In experiment D the material was separated into the following fractions: The plants as they were set out were divided into thirds according to the number of leaves; and at the end of the experiment each plant was divided into five regions of leaves and five regions of stem. The basal three regions were identical with the regions on the plants as set out. Thus the terms "bottom leaves" and "bottom stem" refer to the leaves on the bottom third of the original plant and the portion of the stem to which these leaves were attached. The "next to bottom" and "middle" refer to the successive thirds of the original plant toward the growing point. The "top" fraction consists of the uppermost three leaves and the corresponding portion of the stem, at the end of the experiment. The "next-to-top" portion is the region between the "top" and the "middle" portions and in the ample-phosphorus plants comprises the largest region of the plant.

In all cases where fruit was separated into seed and pulp the following method was used: A fruit was cut in cross section, and the seed, together with the surrounding placental pulp, was removed with a scalpel. This portion was analyzed with the seed; the remaining portion is referred to as pulp. This seems perhaps an unsatisfactory method, but it is exceedingly difficult to separate all the pulp from the seeds in fruits $3/4$ inch to 1 inch in diameter because the seeds are soft and small. In the case of ripe fruits the placental tissue can be freed from the seeds fairly satisfactorily by pressing the pulp surrounding the seeds through a piece of cheesecloth. This was not done in any of the experiments reported here.

In both treatments the lower leaves, which were yellow in color, fell from the plants during the course of the experiment. The bottom

leaf or two died within two weeks after transplanting; after a short time the other lower leaves fell successively until the end of the experiment. Most of the defoliation, however, was on the no-phosphorus plants. Under both treatments it was usual for a leaf to show first a dead region farthest away from the stem. These dead areas increased in size progressively toward the stem. The ventral

Fig. 2.—Plants receiving no phosphorus (left) and ample phosphorus (right)

surface of the leaves of the no-phosphorus plants also had a reddish appearance, probably due to the development of anthocyanin pigments in the subepidermal cells. The ample-phosphorus plants did not show any of these characteristics. It will be seen from Tables 1 and 2 that the plants in the two treatments differed as to weight of plant, amount of linear growth, and the size and number of fruits.

TABLE 1.—*Growth of tomato plants under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

Plants treated with—	Average green weight per plant			Average growth per plant
	Plant as set out	Plant less fruit at end of experiment	Fruit	
	Gm.	*Gm.*	*Gm.*	*Cm.*
No phosphorus	23.78	38.38	13.84	23.08
Ample phosphorus	23.73	179.28	119.09	53.80

TABLE 2.—*Blossom and fruit setting of tomato plants under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

Plants treated with—	Average number of blossoms pollinated per cluster				Average number of fruits set per plant	Average number of blossoms set or partially developed per plant
	Cluster No.					
	1	2	3	4		
No phosphorus	1.90	0.99			0.93	2.67
Ample phosphorus	3.60	4.10	0.93	0.12	3.46	8.22

In May, 1925, the remaining data for experiment D were obtained, which included fresh analyses of the nitrogenous constituents and the saving of the seed from vine-ripened fruits for growth tests, the results of which are given in Table 3.

TABLE 3.—*Growth of plants from the original tomato seeds used in experiment D as compared with growth of plants from seeds produced under ample-phosphorus and no-phosphorus nutrient treatments*

Seeds	Average weight of tops per plant grown in garden soil 31 days		Average weight of plants grown in sand 30 days and watered with nutrient solution 2 (which contained no phosphorus)		Average weight of 1 seed	Germination
	Green	Dry	Green	Dry		
	Gm	*Gm.*	*Gm.*	*Gm.*	*Mgm.*	*Per cent*
Original	0.793	0.080	0.313	0.017	3.30	68.5
No phosphorus	.996	.108	.213	.003	1.68	97.0
Ample phosphorus	1.095	.113	.460	.023	3.54	98.5

At the time experiment D was concluded seed were not saved from the two phosphorus treatments or fresh tissue analysis made. As these data were obtained for experiment F, it was thought advisable in the spring of 1925 to repeat the treatments and obtain these additional data. Two sets of plants were given no-phosphorus and ample-phosphorus nutrient treatment. Seed were saved from ripe fruits of these two treatments and the remaining tissue was used to obtain the data given in Table 7.

A sample of the seed used to start these plants was saved as well as the seed obtained from ripe fruits of the two treatments. It was thought of value to compare the potentialities for growth in these three lots of seed. Germination tests were made under uniform conditions, and the average weight of a single seed was determined. The three lots of seed were sown in pure quartz sand which was watered with a solution containing no phosphorus to determine whether the reduced amount of phosphorus in the seed would have any effect on the amount of growth produced. The seeds were also planted in garden soil to determine whether all lots of seed could produce equally vigorous plants. The growth tests were made in September, 1925.

The experiment was brought to a close because it was apparent that some of the no-phosphorus plants were likely to die from starvation.

In one case the treatment had been carried to such an extent that the plant died. Three plants of the no-phosphorus series were subjected to a treatment of phosphorus supposedly sufficient to restore metabolism, but all died without resuming growth. The roots of the no-phosphorus plants were much shorter than those of the ample-phosphorus plants and were dark brown in color, especially at the ends, whereas the roots of the plants that had had ample phosphorus were white.

Anatomical studies of the stems grown under these different nutrition conditions seem to indicate that there was no significant anatomical difference. The results obtained in Experiment D are shown in Tables 4 to 9, inclusive.

TABLE 4.—*Percentage of phosphorus in the different regions of tomato plants grown under ample-phosphorus and under no-phosphorus nutrient treatments—Experiment D* [a]

Plant regions	Percentage of phosphorus, based on dry weight, in—			Percentage of phosphorus, based on green weight, in—		
	Control plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:						
Bottom	0 302		0. 349	0. 0313		0. 0296
Next to bottom	. 355	0. 109	376	. 0431	0. 0138	. 0358
Middle	. 585	. 105	. 507	. 0887	. 0156	. 0122
Next to top	[b] 978	. 131	. 523	[b] . 1308	. 0154	. 0449
Top			. 162	. 662		. 0211	. 0608
Stems.						
Bottom	262	. 064	. 189	. 0247	. 0077	. 0222
Next to bottom	335	. 047	. 189	. 0274	. 0055	. 0233
Middle	. 598	. 049	. 300	. 0116	. 0052	. 0311
Next to top		. 050	413		. 0051	. 0347
Top		. 190	. 786		. 0187	. 0529
Cluster stems		188	. 543		. 0241	. 0620
Fruit (whole)		. 388	. 825		. 0186	. 0386
Taproot	. 312	. 111	. 200	. 0346	. 0105	. 0206
Secondary root		. 165	. 572		. 0142	. 0654
Fallen leaves:						
Bottom		. 108	. 352		. 1027	. 3356
Next to bottom		. 101	. 323		. 0952	. 3043
Middle		. 109			. 1040	

[a] The no-phosphorus plants were grown 52 days and the ample-phosphorus plants 53 days.
[b] Growing point, which consisted of terminal bud and adjacent leaf which was never more than 4 millimeters in length.

TABLE 5.—*Amounts of dry matter and phosphorus in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D* [a]

Plant regions	Percentage of dry matter in—			Grams of dry weight in—			Milligrams of phosphorus per plant in—		
	Control plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:									
Bottom	10 36	------	8 47	0.559	------	0 146	1.687	------	0.509
Next to bottom	12 24	12.66	9.53	.666	0.054	1 201	2.262	0.059	4.511
Middle	15.17	14.82	8 33	.258	.381	1.449	1.508	.400	7.344
Next to top	[b] 13.37	11.75	8 56	[b] .034	.342	4.790	[b] .335	.449	25.040
Top	------	13 06	9 17	------	.106	521	------	.171	3.449
Stem:									
Bottom	9.47	11.95	11.77	.392	.418	.685	1.027	.268	1.293
Next to bottom	8 18	11 68	12 32	.269	.408	.710	.901	191	1.342
Middle	6.96	10.66	10.39	.038	.242	.752	.225	.11y	2.253
Next to top	------	10.16	8.39	------	.172	1.981	------	.086	8.186
Top	------	9.82	6 72	------	.128	.044	------	.243	.349
Cluster stem	------	12.87	11.43	------	098	.464	------	.184	2.518
Fruit	------	9.56	4.68	------	1.323	9.808	------	5.135	80.900
Taproot	11.10	9.50	10 27	.210	.572	597	.769	.633	1.194
Secondary root	------	8.63	11.43	------	.325	3.890	------	.534	22.240
Fallen leaves:									
Bottom	------	85.04	89.36	------	.655	.549	------	.710	1.929
Next to bottom	------	89.11	84.87	------	.623	.052	------	.630	.017
Middle	------	84.91	------	------	.177	------	------	.193	------
Phosphorus in plant	------	------	------	------	------	------	------	10.00	163.07
Phosphorus in water	------	------	------	------	------	------	------	.29	.29
Total weight	------	------	------	2 43	6 02	27.64	8.714	9.71	162.78
Total phosphorus per plant (1 analysis)	------	------	------	------	------	------	8.74	------	------

[a] The no-phosphorus plants were grown 52 days and the ample-phosphorus plants 53 days.
[b] Growing point, which consisted of terminal bud and adjacent leaf which was never more than 4 millimeters in length.

TABLE 6.—*Percentage of nitrogen in the different regions of tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D* [a]

Plant regions	Percentage of nitrogen, based on dry weight, in—			Percentage of nitrogen, based on green weight, in—		
	Control plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:						
Bottom	2.68	------	1.30	0 300	------	0.111
Next to bottom	3.43	3.27	1.79	.447	0.413	.171
Middle	4.40	3.78	2.30	.714	.562	.192
Next to top	------	4.07	2.96	------	.477	.254
Top	------	4.24	4.34	------	.553	.397
Stem:						
Bottom	1.79	2.24	1.12	.179	.269	.132
Next to bottom	1.98	2.18	.92	.176	.255	.113
Middle	------	2.23	.86	------	.238	.089
Next to top	------	2.36	1.35	------	.240	.113
Top	------	------	3.60	------	------	.242
Cluster stem	------	3.14	2.37	------	.403	.271
Fruit	------	3.84	3.50	------	.184	.164
Taproot	1.82	2.42	1.37	.218	.231	.140
Secondary root	------	2.50	2.80	------	.216	.107
Fallen leaves:						
Bottom	------	2.11	1.20	------	1.820	1.040
Next to bottom	------	2.70	1.23	------	2.310	1.050

[a] The no-phosphorus plants were grown 52 days and the ample-phosphorus plants 53 days.

TABLE 7.—*Fresh tissue analysis for different nitrogenous constituents in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

PERCENTAGE OF NITROGEN BASED ON DRY WEIGHT

Plants	Total	Insoluble	Coag-ulum	Filtrate	Total soluble	Nitrates
As set out:						
Leaves	2.54	1.03	1.04	0.43	1.51	Trace.
Stems	1.48	.50	.23	.76	.98	0 37
No phosphorus.						
Leaves	3.12	1.89	.31	.83	1.23	.77
Stems	1.70	.92	.18	64	.78	.46
Fruit	4.85	2.31	.49	2.02	2 54	.10
Ample phosphorus:						
Leaves	2.63	1.38	.83	44	1 25	.10
Stems	.86	.41	.22	.22	45	.09
Fruit	1.96	.37	.43	1.24	1.59	Trace.

PERCENTAGE OF NITROGEN BASED ON GREEN WEIGHT

As set out:						
Leaves	0.327	0.134	0 134	0 055	0.193	Trace.
Stems	.142	.047	.022	073	.095	0 035
No phosphorus.						
Leaves	619	.379	060	.163	.240	.015
Stems	.274	148	.030	.102	.126	.007
Fruit	.308	.150	031	.128	.158	.001
Ample phosphorus						
Leaves	.283	.148	.089	.047	.135	.011
Stems	.130	.062	.033	033	.068	013
Fruit	.152	.060	.025	.072	.092	Trace.

TABLE 8.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

[Based on dry weight]

AMPLE PHOSPHORUS IN THE NUTRIENT SOLUTION

Plant regions	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicel-lulose
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Stem:					
Bottom	1.68	1 57	0.80	0.74	12.99
Next to bottom	3.25	6.66	.94	1.16	12.87
Middle	4 50	9.33	1 04	1.17	10.11
Next to top	4.06	7 72	1 39	1.78	9.35
Leaves:					
Bottom	.88	2 62	.91	.81	5.00
Next to bottom	.61	1 38	1.22	.77	5.18
Middle	.69	1.40	.97	.56	5.70
Next to top	1.03	1.79	1.11	1.19	5.30
Top	1.33	2.28	1.13	2.06	5.77
Fallen leaves.					
Bottom	.47	.75	.74	.61	5.02
Next to bottom					
Middle					

NO PHOSPHORUS IN THE NUTRIENT SOLUTION

Stem:					
Bottom	2.68	3 72	0.86	0.67	10.35
Next to bottom	5.66	9 32	1.08	.62	11.73
Middle	7.31	10.00	1.45	.72	10.57
Next to top	6.44	9.37	1.16	.37	10.47
Leaves.					
Bottom					
Next to bottom	1.05	1.95	1.15	.21	6.66
Middle	1.14	1.83	1.56	1.02	6.27
Next to top	1.05	2.06	1.36	1.21	6.48
Top					
Fallen leaves:					
Bottom	.89	1.49	.75	.48	5.26
Next to bottom	1.01	1.75	1.02	.63	6.35
Middle	1.00	2.00	1.08	.82	5.93

TABLE 9.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment D*

[Based on green weight]

AMPLE PHOSPHORUS IN THE NUTRIENT SOLUTION

Plant regions	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicel-lulose
	Per cent	Per cent	Per cent	Per cent	Per cent
Stem:					
Bottom	0.196	0.535	0.094	0.087	1.522
Next to bottom	.400	.819	.116	.142	1.587
Middle	.470	.969	.108	.122	1.047
Next to top	.341	.647	.117	.149	.784
Leaves·					
Bottom	.131	.392	.136	.121	.746
Next to bottom	.058	.131	.117	.074	.494
Middle	.058	.117	.081	.049	.475
Next to top	.089	.154	096	.102	.455
Top	.122	.211	.104	.190	.530
Fallen leaves:					
Bottom	.127	.670	.670	.553	4.510
Next to bottom					
Middle					

NO PHOSPHORUS IN THE NUTRIENT SOLUTION

Stem:					
Bottom	0.321	0.446	0.102	0.080	1.238
Next to bottom	.662	1 091	.126	.073	1 370
Middle	.778	1.062	.155	.076	1.127
Next to top	.654	.951	117	.087	1.066
Leaves·					
Bottom					
Next to bottom	.267	.496	.292	.054	1.692
Middle	.169	.271	.231	.152	.931
Next to top	.123	.236	.161	.143	.761
Top					
·llen leaves:					
Bottom	.746	1.265	.634	.410	4.75
Next to bottom	.900	1.550	.900	.559	5.63
Middle	.850	1.698	.912	.695	5.03

EXPERIMENT F

The seed for experiment F was planted February 2, 1924; the seedlings were transplanted to 3-inch pots February 15, 1924, and to 4-inch pots March 23, 1924; and the plants were placed in sand under nutrient treatment on April 11, 1924. The no-phosphorus plants, 89 in number, were sampled June 13, 1924, for preservation, and the ample-phosphorus plants, 40 in number, June 15, 1924. The plants had been under treatment for 63 and 65 days, respectively. A similar number of plants were sampled after 23 and 24 days of treatment in order to determine the rapidity of change in chemical composition. The same divisions of the plant were made in this experiment as in experiment D.

The general outward characteristics during phosphorus starvation were similar to those described under experiment D. Thus the no-phosphorus plants again differed from the ample-phosphorus plants as to chlorophyll appearance, development of anthocyanin pigments, weight of plant, amount of linear growth, and the size and number of fruits. (See Tables 10 and 11, and fig. 3.)

TABLE 10.—*Growth of tomato plants under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

Plants treated with -	Average green weight per plant			Average growth per plant
	Plant as set out	Plant less fruit at end of experiment	Fruit	
	Gm.	*Gm.*	*Gm*	*Cm.*
No phosphorus	35.13	47.60	23.00	21.28
Ample phosphorus	37.43	186.13	206.91	73.30
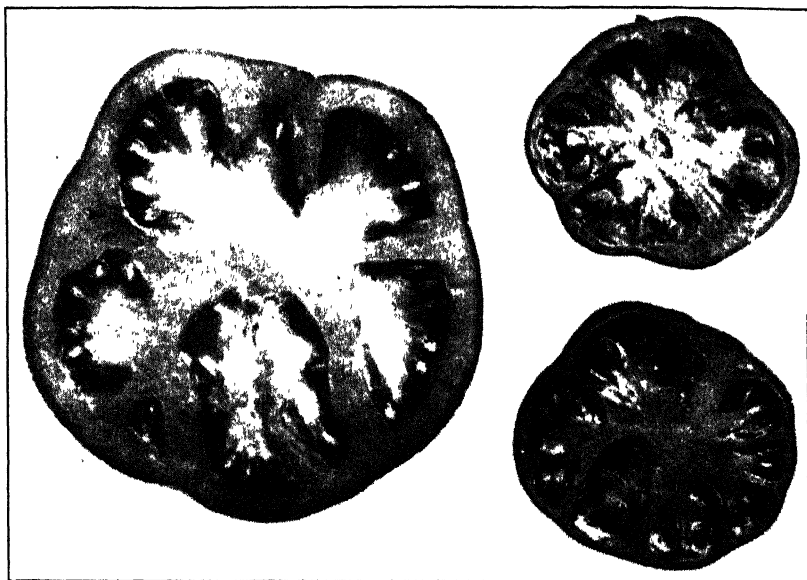
FIG. 3.—Fruits of plants receiving ample phosphorus (left) and no phosphorus (right) in experiment F

TABLE 11.—*Blossom and fruit setting of tomato plants under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

Plants treated with—	Average number of blossoms pollinated per cluster				Average number of fruit set per plant
	Cluster No.				
	1	2	3	4	
No phosphorus	1.55	0.83	0.00		0.87
Ample phosphorus	2.85	1.65	.15	.33	3.70

The experiment was considered complete when two of the no-phosphorus plants had died. The roots were similar in appearance to those already described in experiment D. Six plants of the no-phosphorus series were used in determining what the response

would be to phosphorus applications after phosphorus starvation. The response was quite slow in becoming apparent, but was plainly visible after three to four weeks of treatment. These plants produced new growth, new flowers and fruits, and also ripened some fruits (fig. 4).

The anatomical studies of these stems show a marked increase in the amount of xylem in the ample-phosphorus plants as compared

FIG. 4.—No-phosphorus plants after they had been treated for two months with the ample-phosphorus nutrient solution (experiment F)

with the no-phosphorus plants. In both experiments D and F the fascicular xylem was much more abundant than the interfascicular xylem in all sections.

Seeds from this experiment were saved from vine-ripened fruit in the usual way for separation of pulp from seed by fermentation. The growth tests were made in February, 1925, and the data given in Table 12 were obtained.

Other results obtained in experiment F are summarized in Tables 13 to 18.

TABLE 12.—*Growth of plants from original tomato seeds used in experiment F as compared with growth of plants from seeds produced under ample-phosphorus and no-phosphorus nutrient treatments*

Seeds	Average weight of tops per plant grown in garden soil 34 days		Average weight of plants grown in sand 37 days and watered with nutrient solution 2 (which contained no phosphorus)		Average weight of 1 seed	Germination
	Green	Dry	Green	Dry		
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Mgm.*	*Per cent*
Original	1.98	0.17	0.229	0.036	3.38	69.0
No phosphorus	1.90	.17	.098	.013	2.27	97.0
Ample phosphorus	2.72	.25	.271	.044	4.00	96.5

TABLE 13.—*Percentage of phosphorus in the different regions of tomato plants grown under ample-phosphorus and under no-phosphorus nutrient treatments— Experiment F* [a]

Plant regions	Percentage of phosphorus, based on dry weight, in—					Percentage of phosphorus, based on green weight, in—				
	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:										
Bottom	0.217	0.090	0.164		0.112	0.0172	0.0094	0.0177		0.0113
Next to bottom	.331	.138	.341	0.084	.295	.0301	.0145	.0368	0.0162	.0302
Middle	.543	.210	.514	.100	.313	.0620	.0273	.0605	.0172	.0315
Next to top				.104	.304				.0154	.0342
Top				.168	.483				.0257	.0556
Stem:										
Bottom	.204	.084	.236	.052	.162	.0175	.0102	.0261	.0072	.0208
Next to bottom	.390	.097	.267	.052	.145	.0110	.0103	.0266	.0067	.0187
Middle	.627	.122	.411	.056	.136	.0184	.0115	.0306	.0070	.0176
Next to top				.065	.193				.0074	.0204
Top				.127	.410				.0064	.0133
Cluster stem		.360	.738	.159	.282		.0522	.0506	.0242	.0402
Fruit pulp		b.303	b.538	.197	.488		b.0278	b.0446	.0108	°0289
Fruit seed				.346	.622				.0302	.0508
Taproot		.116	.237	.079	.237		.0127	.0247	.0090	.0236
Secondary root		.186	.376	.093	.399		.0104	0173	.0064	.0256
Fallen leaves:										
Bottom		.103	.157	.091	.146		.088	.131	.0757	.125
Next to bottom		.126	.187	.082	.142		.104	.074	.0682	.116
Middle				.092	.142				.0770	.123

[a] The no-phosphorus plants supplying the data for the second and seventh columns of figures were grown 23 days; for the fourth and ninth columns of figures, 63 days. The ample-phosphorus plants supplying data for the third and eighth columns of figures were grown 24 days, for the fifth and tenth columns of figures, 65 days.

[b] Sample not divided into seed and pulp, but whole fruit analyzed.

TABLE 14.—*Amounts of dry matter and phosphorus in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F* ᵃ

Plant regions	Percentage of dry matter in—					Grams of dry weight in—					Milligrams of phosporous per plant in—				
	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants
Leaves:															
Bottom	7.86	10.33	10.65		9.95	0.556	0.325	0.322		0.265	1.21	0.296	0.527		0.297
Next to bottom	9.07	10.49	10.72	19 20	10 27	1.005	.761	.953	.179	1.302	3.32	1.050	3.250	.151	3.842
Middle	11.43	12.83	11.72	17.08	10.00	.619	.955	2.451	1.066	1.906	3.37	2.005	12.600	1 063	5.966
Next to top				14.60	11.15				.788	8.034				.819	24 42
Top				15.21	11 50				.418	.927				.702	4.479
Stem															
Bottom	8.56	11.96	10.95	13.91	12.71	528	.574	.407	.602	.610	1 08	.482	.961	.313	988
Next to bottom	5.63	10.00	9.99	12.92	12.61	.297	.562	.391	.708	.737	1.16	.546	1.043	.365	1.068
Middle	5.81	9.41	7.42	12.32	12.82	.078	.455	.513	.398	.715	.49	.555	2.109	.224	.972
Next to top				11.37	10 55				.238	2.300				.153	4.440
Top				9.98	6.51				.060	.140				.076	.575
Cluster stem		14 53	13 72	15 16	14 23		.099	.083	.126	.417		.357	.613	.200	1.176
Fruit pulp		ᵇ9.18	ᵇ8.23	8.73	5.92		.549	.343	1.459	8.915		1.662	1.848	2 875	43 500
Fruit seed				5.49	8.16				.344	3.888				1 191	24.180
Taproot		10.84	10.45	11.32	9.95		.331	.430	.713	.799		.384	1.029	.550	1.894
Secondary roots		5.55	4.58	6.87	6 37		.248	.441	.048	1.301		.461	1.656	.602	5.191
Fallen leaves:															
Bottom		85.17	82.23	82.66	85.44		.319	.261	.601	.475		.329	.411	.547	.694
Next to bottom		82.68	78.29	83.44	81.68		.034	.067	.682	1.061		.043	.013	.556	1.508
Middle				84.12	86 80				.241	.226				.221	.321
Phosphorus in plant											10.63	8 17	26 17	10.61	125.51
Phosphorus in water											9.8	.13	.13	34	.34
Total weight						3 083	5.212	6.663	9.270	34.018					
Total phosphorus per plant (one analysis)												8.04	26.04	10.27	125.17

ᵃ The no-phosphorus plants supplying data for the second, seventh, and twelfth columns of figures were grown 23 days; for the fourth, eighth, and thirteenth columns of figures, 63 days. The ample-phosphorus plants supplying data for the third, eighth, and thirteenth columns of figures were grown 29 days; for the fifth, tenth, and fifteenth columns of figures, 65 days.
ᵇ The sample was not divided into seed and pulp, but the whole fruit was analyzed.

TABLE 15.—*Percentage of nitrogen in the different regions of tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F* [a]

Plant regions	Percentage of nitrogen, based on dry weight in—					Percentage of nitrogen, based on green weight in—				
	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants	Control plants	No-phosphorus plants	Ample-phosphorus plants	No-phosphorus plants	Ample-phosphorus plants
Leaves·										
Bottom	2. 45	2. 02	2. 66	--------	1. 72	0. 194	0. 203	0. 284	--------	0. 171
Next to bottom	3 37	2 66	3. 16	2. 90	2. 04	. 305	. 276	. 340	0. 559	209
Middle	4. 55	3 64	4. 36	3 54	2. 46	. 519	. 466	. 514	. 608	. 248
Next to top	--------	--------	--------	3 94	3. 10	--------	--------	--------	. 578	. 348
Top	--------	--------	--------	3. 79	4 56	--------	--------	--------	. 578	. 524
Stem:										
Bottom	3. 92	1 66	1. 82	2 19	1. 57	. 337	. 199	. 200	. 305	. 201
Next to bottom	2. 00	1. 81	2. 11	2. 21	1. 45	. 113	. 192	. 211	. 286	. 184
Middle	2. 85	1 98	2. 89	2. 20	1. 42	. 165	. 187	. 214	. 276	. 183
Next to top	--------	--------	--------	2 18	1. 70	--------	--------	--------	. 247	. 181
Top	--------	--------	--------	2. 72	3 00	--------	--------	--------	. 270	. 196
Cluster stem	--------	3. 33	4. 17	3. 05	2. 63	--------	. 483	. 572	. 462	. 376
Fruit pulp	--------	3. 13	3. 31	3. 05	3. 31	--------	. 285	274	. 168	. 189
Fruit seed	--------	--------	--------	3. 76	3. 87	--------	--------	--------	. 328	. 315
Taproot	--------	1. 68	1. 81	1 87	1 66	--------	. 182	. 189	. 212	. 165
Secondary root	--------	2. 00	2 56	1 75	2 07	--------	. 111	. 117	. 120	. 132
Fallen leaves:										
Bottom	--------	1. 36	1 56	1. 53	1. 57	--------	1. 160	1. 290	1. 270	1. 340
Next to bottom	--------	1. 56	1. 65	2. 20	2. 10	--------	1. 280	1. 300	1. 830	1. 710

[a] The no-phosphorus plants supplying data for the second and seventh columns of figures average were grown 23 days, for the fourth and ninth columns of figures, 63 days. The ample-phosphorus plants supplying data for the third and eighth columns of figures were grown 24 days; for the fifth and tenth columns of figures, 65 days.

TABLE 16.—*Fresh-tissue analysis for different nitrogenous constituents in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

PERCENTAGE OF NITROGEN BASED ON DRY WEIGHT

Plants	Total	Insoluble	Coagulum	Filtrate	Water soluble	Nitrates
As set out:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Leaves	3. 79	2. 28	0 91	0. 52	1 51	0. 45
Stems	2. 04	. 84	. 23	. 79	1. 20	97
Treated 24 days with·						
No phosphorus—						
Leaves	3. 37	2. 67	. 90	. 58	. 70	. 66
Stems	1. 77	. 92	43	. 20	. 85	. 35
Ample phosphorus—						
Leaves	4. 55	3 23	1 15	71	1 32	. 68
Stems	1. 84	. 81	1. 03	. 45	1. 03	. 83
Treated 64 days with.						
No phosphorus—						
Leaves	2. 97	1. 72	. 18	1. 21	1. 25	. 43
Stems	2. 07	. 69	06	1. 39	1. 38	. 32
Ample phosphorus—						
Leaves	2. 97	1. 74	. 69	. 58	1. 23	. 31
Stems	2. 11	1. 37	. 80	. 61	. 77	84

PERCENTAGE OF NITROGEN BASED ON GREEN WEIGHT

As set out:						
Leaves	0. 363	0. 219	0. 088	0. 050	0. 144	0. 044
Stems	. 153	. 063	. 018	. 059	. 090	. 073
Treated 24 days with.						
No phosphorus—						
Leaves	. 441	. 349	. 118	. 077	. 092	. 087
Stems	. 251	. 131	. 061	. 028	. 120	. 049
Ample phosphorus—						
Leaves	. 520	. 370	. 131	. 081	. 150	. 078
Stems	. 245	. 107	. 137	. 060	. 138	. 110
Treated 64 days with:						
No phosphorus—						
Leaves	. 536	. 317	. 032	. 212	. 219	. 075
Steams	. 281	. 094	. 008	. 189	. 187	. 043
Ample phosphorus—						
Leaves	. 327	. 191	. 077	. 064	. 136	. 034
Stems	. 214	. 122	. 097	. 073	. 092	. 101

TABLE 17.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

[Based on dry weight]

AMPLE PHOSPHORUS IN THE NUTRIENT SOLUTION

Plant region	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
	Per cent	Per cent	Per cent	Per cent	Per cent
Stem:					
Bottom	2.85	6.53	1.85	2.36	12.35
Next to bottom	3.21	7.38	2.52	2.99	11.18
Middle	4.61	9.80	3.83	3 54	10.35
Next to top	4.20	10.58	1.71	4.36	9.77
Leaves					
Bottom ᵃ					
Next to bottom	1.03	1.69	.79	1.73	5.57
Middle	1.28	2.11	.88	2.56	6.08
Next to top	1.48	2.55	.86	6.07	6.86
Top	1.14	2.67	1.15	4.76	7.45
Fallen leaves:					
Bottom	.75	1.55	1.09	.79	4.95
Next to bottom	1.23	1.86	1.45	.95	5.03

NO PHOSPHORUS IN THE NUTRIENT SOLUTION

Stem:					
Bottom	4.76	13.02	1.04	2.59	8.21
Next to bottom	6.39	16.93	1.44	1.64	9.66
Middle	8.58	18.90	1.16	1.82	9.55
Next to top	6.91	12.99	1.05	2.23	10.73
Leaves:					
Bottom ᵃ					
Next to bottom	2.90	4.88	.70	.92	5.52
Middle	1.95	2.40	1.07	.65	5.36
Next to top	1.52	2.05	1.16	1.09	6.22
Top	1.39	1.85	.93	3 28	7.41
Fallen leaves:					
Bottom	.62	1.02	1.09	.88	5.37
Next to bottom	1.03	1.04	1.34	.75	5.39

ᵃ There was an insufficient amount of bottom leaves for carbohydrate analysis.

TABLE 18.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and no-phosphorus nutrient treatments—Experiment F*

[Based on green weight]

AMPLE PHOSPHORUS IN THE NUTRIENT SOLUTION

Plant region	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
	Per cent	Per cent	Per cent	Per cent	Per cent
Stem:					
Bottom	0.366	0.833	0.237	0.272	1.582
Next to bottom	.409	.939	.322	.380	1.420
Middle	.595	1.262	.494	.457	1.336
Next top	.446	1.122	.182	.463	1.038
Leaves·					
Bottom ᵃ					
Next to bottom	.104	.172	.081	.176	.566
Middle	.131	.215	.090	.256	.621
Next to top	.166	.287	.097	.680	765
Top	.132	.308	.132	.553	.858
Fallen leaves:					
Bottom	.647	1.330	.981	.675	4.225
Next to bottom	1.014	1.509	1.184	.771	4.195

NO PHOSPHORUS IN THE NUTRIENT SOLUTION

Stem:					
Bottom	0.661	1.805	0.144	0.361	1.136
Next to bottom	.825	2.184	.184	.216	1.247
Middle	1.071	2.360	.145	.227	1.194
Next to top	.784	1.441	.119	.252	1.211
Leaves:					
Bottom ᵃ					
Next to bottom	.559	.938	.135	.177	1.060
Middle	.336	.411	.184	.112	.926
Next to top	.223	.302	.170	.160	.917
Top	.212	.281	.140	.501	1.125
Fallen leaves:					
Bottom	.515	.840	.907	.726	4.44
Next to bottom	.863	.864	1.120	.624	4.50

ᵃ There was an insufficient amount of bottom leaves for carbohydrate analysis.

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EXPERIMENT H

The seed for experiment H was planted July 13, 1924; the plants were placed in sand under nutrient treatment September 11, 1924; the low-phosphorus plants, 26 in number, were sampled for preservation November 28, 1924, and the ample-phosphorus plants, 18 in number, November 29, 1924. The plants had been under treatment 78 and 79 days, respectively. The low-phosphorus plants received approximately 10 per cent of the amount of phosphorus given to the ample-phosphorus plants.

The general characteristics of the ample-phosphorus plants were similar to those of the plants used in experiments D and F, except that they were more mature and ripened more fruits than did the ample-phosphorus plants in experiments D and F. The low-phosphorus plants were smaller than the ample-phosphorus plants and set fewer fruits, and the ventral side of their leaves showed the characteristic anthocyanin pigments. Some of these low-phosphorus plants produced only one fruit, but that one was larger than the average fruits in either the low-phosphorus or the ample-phosphorus group. A few of the low-phosphorus plants did not show as uniform phosphorus limitation as the other low-phosphorus plants. Only one region of these plants which did not show uniform phosphorus limitation was taken for analysis—the next to top—which is so designated in the tables. Samples were taken of all the regions of the other low-phosphorus and ample-phosphorus plants, but the regions designated "bottom," "next to bottom," and "middle" were included in one sample.

Tables 19 to 23, inclusive, give the data obtained in this experiment.

TABLE 19.—*Growth of tomato plants under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

Plants treated with—	Average green weight per plant			Average growth per plant
	Plant as set out	Plant less fruit at end of experiment	Fruit	
	Gm.	*Gm.*	*Gm.*	*Cm.*
Low phosphorus	13.91	128.33	167.06	76.77
Ample phosphorus	13.16	222.27	451.52	132.30

TABLE 20.—*Blossom and fruit setting of tomato plants under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

Plants treated with—	Average number of blossoms pollinated per cluster								Average number of fruits set per plant	Average number of blossoms set or partially developed per plant.
	Cluster No.									
	1	2	3	4	5	6	7	8		
Low phosphorus	1.36	2.10	2.03	0.5	0.6				2.26	6.0
Ample phosphorus	2.14	3.68	3.03	1.68	.5	1.47	.17	.1	6.38	10.5

TABLE 21.—*Percentage of nitrogen and phosphorus in the different regions of tomato plants grown under ample-phosphorus and low-phosphorus nutrient treatments— Experiment H*

[Based on dry weight and green weight]

Plant regions	Nitrogen				Phosphorus			
	Dry weight		Green weight		Dry weight		Green weight	
	Low phosphorus	Ample phosphorus	Low phosphorus	Ample phosphorus	Low phosphorus	Ample phosphorus	Low phosphorus	Ample phosphorus
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Leaves:								
Bottom-middle	2 06	1.42	0.253	0.157	0.153	0.358	0.0191	0.0395
Next to top	2.96	2.45	.322	.228	.155	.500	.0168	.0468
Next to top [a]	3.22	--------	.299	--------	.199	--------	.0186	--------
Top	3.55	3.86	.425	.355	.207	.598	.0249	.0552
Fallen leaves:								
Bottom-middle	2.03	.94	1.840	.830	.079	.220	.0710	.1920
Stem								
Bottom-middle	1.70	.96	.260	.123	.078	.177	.0120	.0228
Next to top	1.51	1.00	.168	.096	.067	.242	.0074	.0222
Next to top [a]	1.86	--------	.164	--------	.162	--------	.0143	--------
Top	--------	--------	--------	--------	.157	.482	.0042	.0151
Cluster stem	2.68	2.03	.217	.280	.199	.476	0322	.0659
Roots	1.95	1.50	.113	.093	.104	381	.0060	.0236
Fruit								
Green seed	3.66	3 83	.249	.211	.316	.645	.0215	.0357
Green pulp	2.86	2.95	.119	.118	.196	.550	.0082	.0215
Ripe seed	3.80	3.62	.285	.274	.615	.646	.0461	.0491
Ripe pulp	3.04	3.23	.109	.143	.394	.619	.0141	.0273

[a] Plants which did not show effects of phosphorus starvation as indicated by the lack of red pigment on the ventral side of leaves.

TABLE 22.—*Fresh-tissue analysis for nitrogen constituents in tomato plants grown under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

PERCENTAGE OF NITROGEN BASED ON DRY WEIGHT

Plants	Total	Water insoluble	Coagulum	Filtrate	Water soluble	Nitrates
As set out:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Leaves	2.94	1 84	0.62	0 55	1.10	0.14
Stems	1.84	.62	.22	.98	1.22	.37
Low phosphorus:						
Leaves	2.85	1 40	.67	.82	1.45	.56
Stems	1.91	.91	.22	.85	1.01	47
Fruit	3.07	1.27	.15	1.68	1.80	---------
Ample phosphorus:						
Leaves	2.31	1.45	.43	.53	.87	.13
Stems	.95	.50	.20	.30	.45	.11
Fruit	2.79	1.67	.05	1.04	1.12	---------

PERCENTAGE OF NITROGEN BASED ON GREEN WEIGHT

As set out·						
Leaves	0.452	0.288	0.092	0.082	0.164	0.021
Stems	.225	.075	.027	.120	.150	.045
Low phosphorus:						
Leaves	.334	.164	.079	.096	.170	.065
Stems	.222	.105	.025	.099	.117	.055
Fruit	.197	.080	.009	.109	.117	---------
Ample phosphorus:						
Leaves	.242	.151	.045	.055	.091	.013
Stems	.121	.064	.025	.039	.057	.012
Fruit	.176	.169	.003	.007	.007	---------

TABLE 23.—*Percentage of carbohydrates in tomato plants grown under ample-phosphorus and low-phosphorus nutrient treatments—Experiment H*

PERCENTAGE OF CARBOHYDRATES BASED ON DRY WEIGHT

Plant regions	Carbohydrates in low-phosphorus plants					Carbohydrates in ample-phosphorus plants				
	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
Leaves:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Bottom_ _____										
Middle_____	1. 59	2. 49	1. 18	1. 84	5. 38	1 00	2. 06	0. 89	0. 89	4 95
Next to top_____	2. 29	3 29	. 89	5. 50	7. 09	1. 16	2. 04	. 76	1. 56	7. 20
Do.ᵃ_____	1. 36	3 12	1. 43	4. 66	5 87					
Fallen leaves.										
Bottom_____										
Middle_____	. 98	2. 87	1. 22	1. 08	5 29	. 79	2. 17	1. 02	1. 04	4. 93
Stem.										
Bottom_____										
Middle_____	4. 48	9. 07	1. 73	1. 88	11. 49	2. 84	5. 74	. 84	. 94	12 13
Next to top_____	8. 12	12 50	1. 18	1. 25	11. 10	3. 80	6. 38	1. 08	1. 05	10. 12
Do.ᵃ_____	4. 19	7 87	. 94	1. 47	8. 86					

PERCENTAGE OF CARBOHYDRATES BASED ON GREEN WEIGHT

Plant regions	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose	Reducing sugars	Total sugars	Dextrin soluble starch	Starch	Hemicellulose
Leaves·										
Bottom_____										
Middle _____	0. 199	0. 315	0. 147	0. 231	0. 672	0 109	0 227	0. 097	0 097	0. 539
Next to top ____	250	361	. 096	. 596	769	. 108	. 193	. 071	. 146	. 671
Do.ᵃ_____										
Fallen leaves.										
Bottom_____										
Middle_____	886	2 610	1. 113	. 982	4. 760	686	1. 887	908	. 908	4. 280
Stem:										
Bottom_____										
Middle_____	. 685	1. 383	. 264	. 288	1. 752	364	. 734	. 109	. 120	1. 556
Next to top_____	. 902	1. 383	. 130	. 139	1. 232	. 364	. 607	. 103	. 101	. 972
Do ᵃ_____										

ᵃ Plants which did not show effects of phosphorus starvation as indicated by red pigment on under side of leaves.

RIPENING EXPERIMENT

It seemed advisable to make some analyses of tomatoes at different stages of growth to determine the distribution of phosphorus in the fruit. Plants for this experiment were set in the greenhouse bench during the spring of 1924 and in a different bench in the summer of 1924. To insure a difference in soil fertility in the two tests, the soil used in the second test was supplied with a liberal quantity of sheep manure and steamed bone meal. These two experiments are referred to according to the time the fruit ripened as April–June and August–September (Table 24).

There is difficulty in determining the stages of ripening in the tomato; the following method, however, was used successfully. The first stage was based on the date of pollination and the size of the fruit—3 to 5 mm. in diameter three to five days from pollination. It was necessary to take the date into consideration so that no fruits would be included which had started ovary development, but would never produce a mature fruit. The second stage was based wholly on the size of the fruit, that is, ¾ inch to 1 inch in diameter. The fruit of this stage was approximately three weeks old. The number of days from pollination to the ripening of the fruit fluctuates so much as to make such a basis of questionable value in the more mature

stages. The other three stages, therefore, were determined according to the consistency of the fruit as shown by testing in the palm of the hand for hardness and according to the color of the fruit. The more advanced stages were hard green, soft green, and ripe. The method used in separating fruits into pulp and seed and surrounding pulp is described in experiment D.

TABLE 24.—*Phosphorus content of tomatoes at different stages of growth—plants grown in garden soil*

Material and stages	Percentage of phosphorus based on—				Total amount of phosphorus per fruit		Total weight per fruit			
	Dry weight		Green weight				Dry weight		Green weight	
	April-June	August-September	April-June	August-September	April-June	August-September	April-June	August-September	April-June	August-September
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Mg.*	*Mg.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Pulp.										
First stage (3 to 5 mm. in diameter)a										
Second stage (¾ to 1 inch in diameter)	0 571	0 561	0.0410	0.0490	1.55	1.27	0.272	0.227	3.79	2.65
Third stage (hard green)	.465	.589	.0378	.0280	11.38	10 81	2.452	1.835	81.08	38.51
Fourth stage (soft green)	.361	.646	.0157	.0219	21 85	20 17	6 072	3 123	138.97	91.73
Fifth stage (ripe)	.362	.576	.0174	.0288	26.18	24.86	7.232	4.316	150.24	86.14
Seed and surrounding pulp·										
First stage (3 to 5 mm. in diameter)	1.570	1 400	.0017	.0015	.053	.054	.0035	.0038	.0304	.0348
Second stage (¾ to 1 inch in diameter)	.793	.805	.0244	.0261	1.205	1.123	.152	.1394	2 44	2.15
Third stage (hard green)	.683	.666	.0380	.0348	7.957	5.350	1 165	.803	20.98	15.44
Fourth stage (soft green)	.582	.633	.0398	.0405	17.17	10.98	2 951	1.735	43.25	27.05
Fifth stage (ripe)	.567	.777	.0372	.0309	16.31	15.01	2.876	1.932	43 78	48.61

a Pulp and seed were not separated in this sample

The changes in cellular structure during the ripening process are shown by the accompanying drawings (figs. 5 and 6). A fruit may be divided into the carpel, the placental region, and the developing seed. The carpel and the placental region undergo changes in size of cells and also a progressive disintegration of the cell walls. At the time of pollination the ovules are not surrounded by placental tissue; but there is a very active division and enlargement so that in a very short time, probably not more than a week after pollination, the ovules are surrounded by the placenta. The cells composing the placental outgrowth begin disintegration by becoming gelatinized. This process begins, in some cases at least, within three weeks after pollination. Disintegration occurs next in the more compact outer portions of the placenta below the level of attachment of the seeds. Finally some of the cells of the carpel walls disintegrate also. In both of these regions the cells enlarge enormously before disintegrating. Little or no change takes place in the vascular region, as these cells can be found intact in a fully ripened

fruit. Comparisons between the fruits of different ages, however, should be made primarily on the basis of size of cells, because different fruits as well as the same fruit, may differ as to the thickness of the carpel wall.
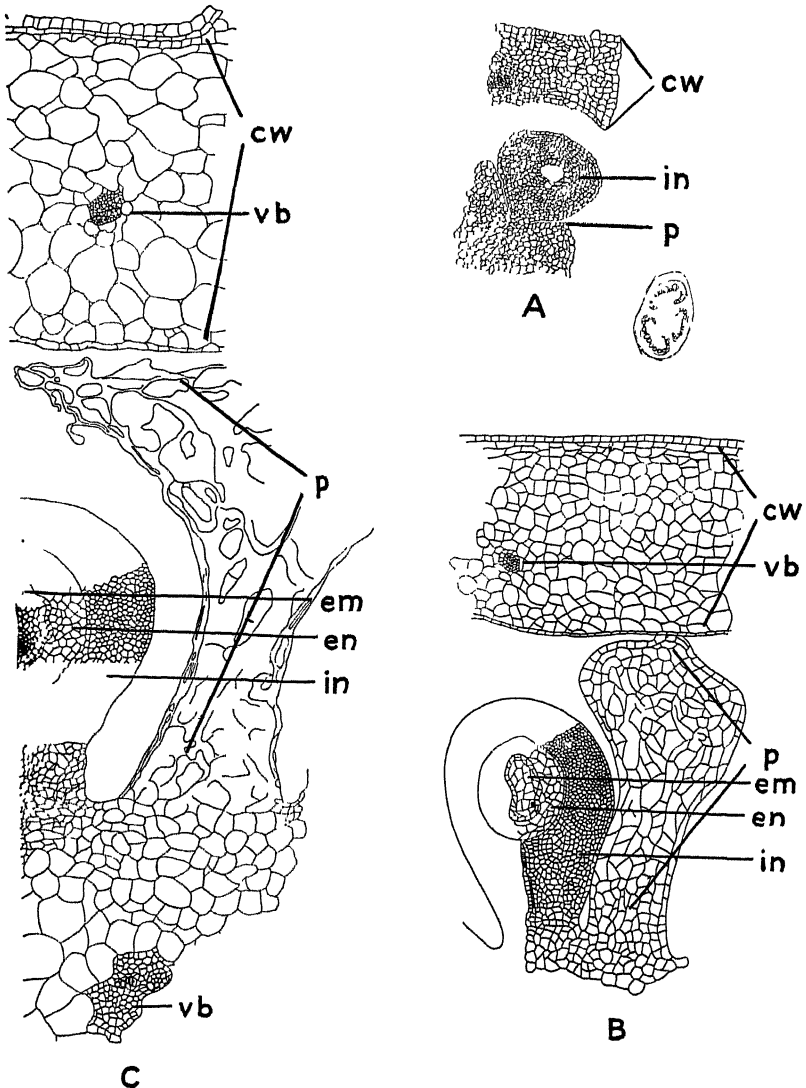
Fig. 5.—Camera-lucida drawings of sections of tomato fruits. × 66.3. A, fruit three days after fertilization, B, fruit approximately 10 days after fertilization; C, fruit ¾ inch to 1 inch in diameter. *cw*, carpel wall; *en*, endosperm; *em*, embryo; *in*, integument; *p*, placenta; *vb*, vascular bundle

Changes also occur in the seed as the fruit ripens. Souèges (*13*) has made the most extensive study of the development of the seeds of the family Solanaceae. The similarity in the development of the various members of this family is quite noticeable. This investi-
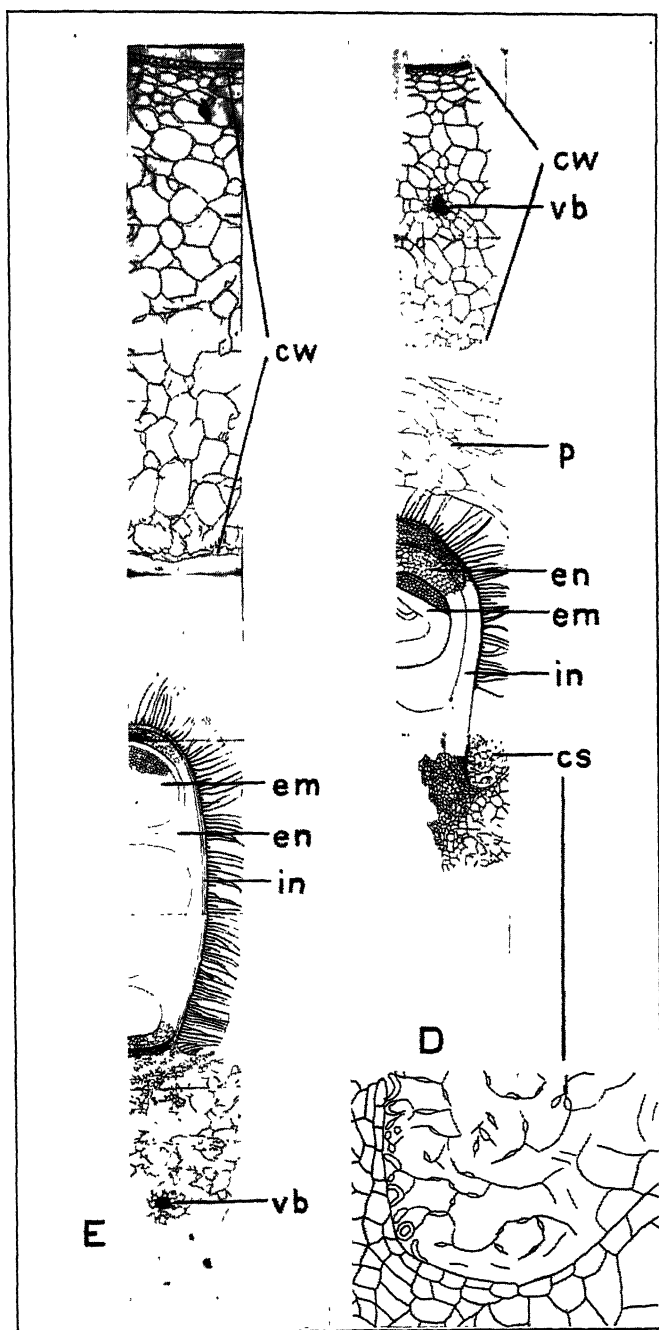
FIG. 6.—Camera-lucida drawings of sections of tomato fruits. ✕ 23.6. D, green fruit before ripening; E, ripe fruit. *cs*, cross-section view of spines (enlarged below); *cw*, carpel wall; *en*, endosperm; *em*, embryo; *in*, integument; *p*, placenta; *vb*, vascular bundle

gator has studied the development of the macrogametophyte very carefully. Up to the time of fertilization the integument is composed of cells uniform in size and shape, but soon afterwards these differentiate into the external layer (assise externe) and the internal layer (assise interne) of the integument. Between these two layers are two regions called the internal and external zones (zone externe and zone interne). The nucellus is completely digested, so that the macrospore mother cell lies in direct contact with the internal layer of the integument. Some have considered this layer as the outermost layer of the nucellus.

The outer layer of integument cells enlarges as the seed matures and longitudinal thickenings of cellulose are formed in these walls. These thickenings increase in size, turn brown, and the intervening thin portions of the cell walls disintegrate, so that the thickenings appear to be spinelike. Figure 6, D shows on an enlarged scale the character of the cellulose thickenings from two aspects. The cross-section view near the funiculus shows where the thin cell walls have disappeared in some cells, and in other cells the thin wall can still be seen.

Tschirch and Oesterle (*15*) portray the same structures for the mature seed for Capsicum and Datura. In Datura the layer referred to by Souèges as the internal layer of the integument is called nucellus, while the same layer is referred to as the perisperm by Winton and Moeller (*18*) in their figure of the mature seed of *Lycopersicum esculentum*.

Groth (*4*) has made a study of the structure of the cells found in the "skin" of the fruit of various types of tomatoes.

DISCUSSION

The distribution of phosphorus in a starved plant and its distribution in a plant developed with an ample supply show striking similarities. The regions in which the highest percentages of phosphorus (based on dry weight) occur are the same, under both treatments (the fruit, cluster stems, top leaves, and top stem). In some earlier analyses of tomato plants grown in garden soil, in which the plants were more finely subdivided, it was found that the nodes and leaflets had a greater percentage of phosphorus than the adjacent regions— the internodes and midrib of the compound leaf. These facts seem proved by all the data, but especially by the percentages based on dry weight. The percentages of phosphorus in the plants receiving an ample supply of phosphorus show an almost steady increase from the bottom to the top regions of the stem. The plants grown in the nutrient solution lacking phosphorus do not show such an increase in phosphorus content, but approximately the same percentage of phosphorus is found in all the regions of the stem from the bottom to the next to top. This naturally leads to the conclusion that high percentages of phosphorus are associated with regions of rapid growth. In the plants grown in the absence of phosphorus the supply is so limited that only in the very top part of the plant is there active growth. It would be of interest to know just what the difference is between the organic and inorganic forms of phosphorus in such embryonic regions. If one could completely separate the embryonic regions for purposes of analysis it might be that these regions even

under different treatments would be found to have equal percentages of phosphorus, at least there might be less relative difference than these tables show.

As all the regions of high phosphorus content are areas which have developed subsequent to the plant's being transplanted to sand, there is no doubt that phosphorus is subject to reutilization during starvation or a time of limited supply. The regions in question are the fruit, cluster stems, top leaves, top stem, next-to-top leaves, and next-to-top stem. The total amount of phosphorus in all regions shows that, regardless of the treatment, approximately one-half of the total phosphorus is located in the fruit. The leaves in the bottom three regions should contain the same total amount of phosphorus at the end of the experiment as at the beginning if there is no reutilization of the element. The mere decrease in the percentage of phosphorus would not prove that the phosphorus had been removed, as the decrease in the percentage of phosphorus might be accompanied by an increase in the total dry weight. In experiment D, 68 per cent of the phosphorus has been removed from the next-to-bottom leaves, and in the same region in experiment F, 78 per cent has been removed. The leaves fell from the bottom region within a short time after transplanting—too soon to permit of the removal of such a large proportion of the phosphorus.

The nonremoval of phosphorus from these lower leaves might indicate that phosphorus was not transported with rapidity in the plant. Plants which had been phosphorus-starved for two months showed very slow recovery or nonrecovery when phosphorus was added to the nutrient solution. This is further evidence that the rate of movement of phosphates is dissimilar to that of nitrogen, as additions of nitrates can be recognized by plant performance within a few days.

The results obtained by André (*2*) show that phosphorus is redistributed to a greater extent than most of the other minerals. This property of great reutilization may be a contributing reason why plants have a smaller total amount of phosphorus than of nitrogen, potassium, and some other minerals. The results obtained by Le Clerc and Breazeale (*9*), Wilfarth, Römer, and Wimmer (*17*), Schulze and Schütz (*11*), and Wehmer (*16*) are suggestive of reutilization of the mineral elements, although the proof is not so conclusive as that presented by André.

Under the two treatments there were notable differences in the number of blossoms opened, the number of blossoms setting fruit, and the proportion of these fruits which continued to grow. Blossoms frequently turn yellow and fall from the plant before blooming. The data show that this is much more common in the plants grown in the absence of phosphorus than where ample phosphorus is present. Inasmuch as blossoms require cell division for their development it would seem as if the limited supply of phosphorus was not sufficient to provide the inorganic and organic phosphorus necessary. Even with the reutilization of this element the demands are not fulfilled.

As flower primordia are formed strikingly early in the life of a plant, it seems evident that these plants should have had the same number of flower buds, at least in the first few clusters. One should consider the possible effect of a limited supply of phosphorus at an early stage on the number of bud primordia formed. The phos-

phorus content of potting soils might thus influence the number of buds on the early clusters of a plant. When the possibilities of reutilization as well as the smallness of the quantities of phosphorus needed in such embryonic regions are taken into consideration, it seems unlikely that the number of floral buds would be reduced on this account.

The fruits which were immature at harvest were of two kinds: First, those which had made continuous growth; and secondly, those which had grown for only a short period after fertilization. It is likely that the cessation of growth in the latter was due to insufficient food supply. Single mature fruits were very prevalent on the plants grown in the absence of phosphorus; therefore it would seem that one fruit could cause a sufficient deficiency of phosphorus to prevent the development of the other fruits. The fruits which continued to develop were almost invariably the ones which were pollinated first. Priority of pollination perhaps accounts to a certain extent for the variation in the size of fruits within a single cluster. In ordinary plants grown in greenhouses some of the fruits of a cluster are larger than others. There is some variation in the position of the larger fruits, but usually they result from the earlier-pollinated flowers. Some of the flowers produce ovaries which develop only a few centimeters in diameter. Usually the calyx develops so as to accentuate the smallness of the ovary. A study of such factors as heredity, pollen, position of fruit in cluster, and position of cluster on plant, as affecting the size of the fruit, would be of interest.

It is commonly stated that phosphorus is essential to seed formation. Just what interpretation should be given to this statement is uncertain. Phosphorus is essential to the formation of cells and thus would be necessary to the formation of seed or any other structure of the plant, whether fruit or vegetative part. It is doubtful if phosphorus is mainly essential only to the formation of the seed portion of the fruit.

Such statements are probably based on analyses of the fruits of plants such as wheat and corn, which are commonly considered seeds, but the presence of the outer coat, which is a portion of the carpel wall, usually called a fruit coat, causes them to be classified as fruits. These fruits have rather a large amount of phosphorus, but just how much is in the seed is not known. Since the fruit coat of wheat is very thin and constitutes a very small percentage of the total wheat fruit, it seems likely that phosphorus might be more essential to its seed formation than to the seed formation of a tomato, the nonseed portion of the fruit of which has a dry weight more than double that of the seed portion. The tomato is not a very satisfactory fruit for determining whether the greatest amount of phosphorus is found in the seed, the carpel wall, or the placental pulp, because it is difficult to separate these portions from one another in a quantitative manner. However, even with this error which increases the amount of phosphorus in the portions classified as seed in this paper, there is some doubt as to the statement that phosphorus is essential only to seed formation. A plant such as a legume or some member of the cabbage family the fruit parts and seed parts of which are distinctly separate would be more desirable for this study.

An examination of Figure 3 shows that both treatments seem to produce normal seed. The germination tests and the growth of seeds in soil show that seeds from plants grown in the absence of phosphorus are able to reproduce the plant almost as efficiently as seeds from plants grown in the presence of an ample amount of phosphorus. The analyses based either on the percentage or on the total amount of phosphorus show that the seed suffers just as much as the pulp under limited phosphorus nutrient conditions.

Phosphorus is just as essential to the formation of pulp as to the formation of seed. The figures referred to in the previous paragraph indicate that the effects of phosphorus starvation on the development of pulp are as marked as its effects on the seed. The seed is mature and differs only in size; the pulp is decreased in amount both in the carpel walls and in the central placental region. The differences produced by the two treatments are of such great magnitude that one might suspect that the plants were of different varieties. A tomato whether grown in garden soil or in a nutrient solution containing an ample amount of phosphorus or a nutrient solution containing no phosphorus has a larger total quantity of this element in the pulp than in the seed. Of the total amount of phosphorus in a fruit, the percentage found in the seed varies little with treatment, being 29.3 per cent in the plants grown in the absence of phosphorus, and 35.7 per cent in the plants grown in the presence of an ample supply of phosphorus. It should be remembered, however, that the fruits produced by the no-phosphorus plants are smaller and have fewer seeds than those produced by the ample-phosphorus plants. A fruit grown in normal garden soil either with or without the applications of steamed bone meal has a greater total amount of phosphorus in the pulp than in the seed. This is because the pulp has a greater dry weight than the seed, and not because it has a greater percentage of phosphorus. This is true not only of ripe fruits, but also of all stages of green fruits in which it is possible to separate the seed and the pulp. The presence of phosphorus in such large quantities in the pulp at all times regardless of age or phosphorus nutrient treatment shows that phosphorus is essential not only to seed formation, but also to the formation of pulp.

It is of interest in connection with this discussion to consider some of the processes in the formation of fruit. The ripening process seems to include a change of carbohydrates of high molecular weight to sugars, an increase in dry and fresh weight, an enlargement of practically all cells, and the disintegration of the cell walls. The chemical changes during ripening have been very thoroughly investigated by Albahary (*1*) and Sando (*10*). The enlargement of the cells would not require large quantities of phosphorus, still the origin of such cells would require cell division in an embryonic region. Such a region would naturally be high in phosphorus whether it was to produce ovule or vegetative cells. After these cells are formed there is a great increase in their size, accompanied by an increase in the amount of phosphorus present. Thus maturation in the tomato is accompanied by progressive increases in the amount of phosphorus. The data in the ripening experiment show that the tissue contains the highest percentage of phosphorus in the embryonic stage. If tissue of an earlier stage could be obtained in sufficient quantities for

analysis, probably it would be found to contain a still higher percentage of phosphorus. The presence of an ample supply of phosphorus at such an early stage must have an important influence on the size of fruit which will develop. Some cell division must occur after the 3 to 5 mm. stage, as there are embryonic regions in these fruits and the placenta has not yet grown around the seed.

The weight of a single seed of a no-phosphorus plant is less than that of a single seed of a full nutrient solution plant. This result is in agreement with the results of similar experiments conducted by Hellriegel, Wilfarth, Römer, and Wimmer (7) with barley, in which the weight per seed increased with the amount of phosphorus added to the soil. Seeds from the no-phosphorus plants seem able to pass through the seedling stage and become independent plants. The lighter seeds seem to produce less stocky plants, a circumstance which might affect yield and earliness of fruit, as has been shown by previous workers with seeds of different weights. Seeds from plants grown under the two treatments when grown in sand cultures without phosphorus showed that the difference in phosphorus reserve affected the amount of growth.

Statements have been made to the effect that some plants can be supplied with sufficient phosphorus in the early stages of growth and are benefited by maturing in a solution lacking phosphorus (3). This investigation shows that a tomato plant can not absorb sufficient phosphorus from a greenhouse potting soil in eight weeks to supply its needs for continued development through an equal length of time without an additional supply of phosphorus. The effects of starvation were noticeable in some of the plants that had been deprived of phosphorus only two or three weeks. Gericke (3) worked with the wheat plant, which differs from the tomato in that it has a definite period of vegetation and a definite period of fruiting. In the tomato, fruit formation and production of vegetative growth may occur at the same time. As plants will absorb greater quantities of an element than they need for growth (8), it is likely that such excess of phosphorus plus the phosphorus removed from the old dying parts is sufficient to mature the fruit of the wheat in a nutrient solution lacking phosphorus.

Hartwell (5, 6) has shown that plants differ as to their capacity to use the phosphorus of the soil. Carrots and tomatoes were able to obtain phosphorus from the soil for growth under conditions under which turnips, beets, and rape would be unable to grow satisfactorily without the addition of phosphate fertilizer.

The recovery of the phosphorus in the no-phosphorus plants was considered satisfactory, except in the case of the plants treated 24 days in experiment F. This large error may have been due to the greater amount of phosphorus in the newly formed roots and thus a greater loss in collecting this sample than there was in collecting the 64-day sample. It should be kept in mind that there were the following unavoidable very small errors in recovering the phosphorus: The errors of analysis; flower buds lost through abscission; loss of roots in removal from sand; and loss after blooming of such parts of the flower as the corolla, style, and stamens. There seems to have been no loss of phosphorus from the plant to the surrounding medium, which was much lower in phosphorus content.

In the absence of a sufficient amount of phosphorus one would expect a disorganization of the parts of the cell and consequently a disorganization of its normal functions. Cells in such condition are apparently unable to form chlorophyll to replace that lost through decomposition. Another circumstance which might explain chlorophyll deficiency is the accumulation of the end products of the reaction—sugars. As the accumulation of sugars would tend to stop the synthesis of sugars, it is unlikely that chlorophyll would be formed if it were not going to be used. These are possible explanations of the decreased chlorophyll content of the tomato leaves at the end of the experiment.

Some plants, such as the tomato, when grown under conditions of low nitrogen supply, develop certain marked external characteristics. Such plants are not infrequently spoken of as "nitrogen-starved" or as "carboyhydrate-high" plants. Because of the frequency with which these terms are used, it is easy to overlook the fact that a similar characteristic appearance may be produced by the deficiency of other essential elements or by other factors affecting growth. From the description of the plants under "Data and results," it would seem that phosphorus and nitrogen starvation have some external characteristics in common. Among these might be mentioned the stiffening of the stem; decrease in chlorophyll, as shown by yellow leaves; and the appearance on the under side of leaves of anthocyanin pigments.

The analyses giving the amounts of the elements in percentage show that embryonic regions, such as the fruit, cluster stem, top leaves, and top stem, are not only high in phosphorus, but also in nitrogen. The regions of highest nitrogen are, however, slightly different from the regions of highest phosphorus, being the top leaves instead of the fruit and cluster stems. As previously explained, these embryonic regions are high in protein, so one would expect a high percentage of the elements such as phosphorus and nitrogen, which compose the proteins. When a comparison is made of the percentages of nitrogen in the plants having had the two treatments the facts found are less easy to explain. Plants grown in sand in the absence of phosphorus usually have a greater percentage of nitrogen than plants having had an ample supply of phosphorus. The plants grown in the absence of phosphorus show a decrease in the percentage and also the total amount of coagulable nitrogen as compared with the total coagulable nitrogen in the plants grown in the presence of an ample supply of phosphorus. The increase in the percentage of nitrogen in the no-phosphorus plants is confined to the water-soluble forms of nitrogen, excluding nitrates.

Phosphorus starvation seems to affect the carbohydrates primarily as to the amount of sugars found in the plant, but its effect on the other carbohydrates is not very consistent. The quantity of reducing sugars and the total quantity of sugars increased greatly in the plants grown in the absence of phosphorus as compared with the quantities in the plants grown in the presence of an ample amount of phosphorus. These increases in sugars were usually accompanied by a lower percentage of starch. If, as has been previously shown, starvation interferes with the coagulable-nitrogen synthesis it is natural that there should be an increase in carbohydrates. It should be noted,

however, that phosphorus starvation and nitrogen starvation affect the tomato plant differently, as the latter brought about a predominant storage of starch.

The plants grown in the absence of phosphorus almost trebled their dry weight during both experiments. This seems in harmony with the increase in percentage of carbohydrates. It does not seem, however, that this would entirely account for this increase.. Some of this weight must have been due to cellulose and lignin substances not determined.

As experiment D was conducted in the fall and experiment F in the spring and as they were continued different lengths of time there were a number of variations in the results obtained. Thus, in experiment F there is a greater difference in the carbohydrates in the plants under the two treatments than is shown in experiment D. This might have been expected inasmuch as these plants were grown in the spring and the experiment extended over a longer period of time than did experiment D. The difference in the time of year and length of time the experiments were conducted seems to have exerted an almost negligible effect on the phosphorus content of the plants.

This study has been planned primarily to increase our knowledge of the effect of phosphorus starvation on the growth of the tomato plant, yet there are suggestions which are of practical significance.

Since the tomato is a plant which may normally have at the same time flowers and green fruit at various stages of maturity, it would seem essential to supply the plant with a continuous supply of phosphorus. This is necessary during fruit formation if good-sized fruits are desired. It is also advisable, inasmuch as about one-half of the phosphorus of the plant is found in the fruit. It should be possible to detect phosphorus-starvation conditions in the tomato by the symptoms here described.

SUMMARY

These studies show that phosphorus starvation has the following effects on a tomato plant: (1) The percentage of phosphorus is decreased in all parts of the plant; (2) there is a reutilization of the phosphorus; (3) regions of growth contain the highest percentage of phosphorus; (4) there is a decrease in the size of fruit; (5) the dry weight of the plant is trebled in two months; (6) the coagulable nitrogen content is decreased; (7) the percentage of total nitrogen increases, the increase being primarily in the water-soluble forms of nitrogen, excluding nitrates; (8) there is a marked increase in the percentage of sugars.

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CRITICAL TESTS OF TETRACHLORETHYLENE AS AN ANTHELMINTIC FOR FOXES [1]

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INTRODUCTION

In a recent paper Hall and Shillinger (*2*) [3] called attention to the promising results obtained by them in critical tests of tetrachlorethylene as an anthelmintic for dogs. They found the drug very effective against hookworms and ascarids. In a later paper these authors (*3*) published protocols of tests on about 60 dogs. Their experiments indicated that the effective dose was 0.2 c. c. per kilo. At this dose rate tetrachlorethylene removed 195 of 196 hookworms in 9 infested dogs and all the ascarids—32—in 4 infested dogs. Dogs survived doses of 3, 5, 10, and 15 c. c. per kilo, which indicates that the margin of safety is high in the absence of contraindications. Of 64 dogs treated with doses ranging from 0.2 to 0.6 c. c. per kilo, 3 died from 1 to 3 days after treatment. These three animals were showing clinical symptoms of distemper at the time of treatment. The authors concluded that the contraindications for tetrachlorethylene probably are identical with those for the related compound, carbon tetrachloride. Liver lesions were noticed after the administration of tetrachlorethylene.

Schlingman (*5*) performed critical tests of tetrachlorethylene on 31 dogs, mainly puppies 3 to 4 weeks old. In these tests the doses varied from 0.2 to 10 c. c. per kilo, and the drug was 100 per cent effective against hookworms in all cases and 87.7 per cent effective against ascarids in 25 cases. His findings indicate that the safety of tetrachlorethylene for puppies is higher than that of carbon tetrachloride. He also found that tetrachlorethylene apparently is not so likely to cause inhalation intoxication as is carbon tetrachloride when the containing capsules accidentally break in administration.

Since tetrachlorethylene has given promising results in tests on dogs, it was considered advisable to conduct critical tests of this new anthelmintic on foxes. The results of these experiments are reported in this paper. Of the 26 foxes used in these tests, 16 were pups from 7 to 9 months of age and the other 10 were 1 year old or more.

ANTHELMINTIC TESTS

In the experiments on foxes the animals were given chemically pure tetrachlorethylene after a fast of 16 to 20 hours. The drug was given in hard gelatin capsules, administered by means of a balling gun consisting of a rubber tube about 2 feet long and a rattan plunger.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 2
Jan. 15, 1927
Key No. Q-5

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The mouth of the animal was restrained by means of a mouth clamp, an instrument consisting of a wooden gag and a clamping device for holding the jaws down tight on the gag. The rubber tube was passed three-quarters of the way down the esophagus and the capsules containing the drug were then forced down the tube by means of the plunger.

The animals were fed two to three hours after dosing, and while on the tests were given a ration once a day consisting of raw ground beef hearts and liver, baked cereals, whole milk, and bone meal

After treatment the foxes were confined in small individual cages with 1-inch-mesh wire bottoms, set over galvanized-iron collecting pans. All worms passed were collected once a day, identified, and counted. From 48 to 72 hours after dosing the animals were killed, either with an intracardial injection of strychnine sulphate or by stunning with a blow on the head, which was promptly twisted so as to dislocate the atlanto-occipital articulation. The worms present when the post-mortem examination was made were identified and counted, and gross pathological conditions were noted, especially those due to the anthelmintic.

Tests conducted to determine the anthelmintic efficiency of tetrachlorethylene when given without purgation are reported in Tables 1, 2, and 3.

TABLE 1.—*Anthelmintic efficiency of tretrachlorethylene given without purgation at a dose rate of 0.2 c. c. per kilo*

Fox No.	Weight	Number of worms passed		Number of worms found post-mortem		Efficacy in expelling—	
		Hook-worms	Ascarids	Hook-worms	Ascarids	Hook-worms	Ascarids
	Kilos					*Per cent*	*Per cent*
1325	3. 0	1	0	0	0	100	
1326	3. 2	3	0	0	0	100	
1327	2. 8	0	0	0	0		
1328	2 8	20	3	0	0	100	100
1329	3. 4	5	7	0	0	100	100
1330	3. 2	27	0	2	0	93	
1337	4 4	5	0	0	0	100	
1338	4. 2	0	0	0	0		
1339	4. 6	9	0	0	0	100	
1346	4. 7	2	0	0	0	100	
1347	3. 7	3	4	0	0	100	100
1348	3 0	10	1	0	0	100	100
Total		85	15	2	0	97 7	100

All the foxes in the foregoing tests were constipated except Nos. 1337 and 1339, and after treatment these passed feces that were soft and semiliquid. Fox No. 1325 was showing marked clinical symptoms of lungworms when treated. On post-mortem a heavy infestation of *Capillaria aerophila* was found, and a chronic inflammation of the mucosa of the trachea, bronchi, and bronchioles, and in these parts there was an abundance of a very thick exudate.

TABLE 2.—*Anthelmintic efficiency of tetrachlorethylene given without purgation at a · dose rate of 0.3 c. c. per kilo*

Fox No.	Weight	Number of worms passed		Number of worms found post-mortem		Efficacy in expelling—	
		Hook-worms	Ascarids	Hook-worms	Ascarids	Hook-worms	Ascarids
	Kilos					*Per cent*	*Per cent*
1340	3 7	23	0	0	0	100	
1341	4.8	2	0	0	0	100	
1342	4.3	0	0	0	0		
1343	4.1	18	1	0	0	100	100
1344	3.3	4	0	0	0	100	
1345	3.4	12	1	0	0	100	100
Total		59	2	0	0	100	100

All the foxes were constipated except Nos. 1343 and 1345, the feces of which, passed after treatment, were soft and semiliquid.

TABLE 3.—*Anthelmintic efficiency of tetrachlorethylene given without purgation at a dose rate of 1 c. c. per kilo*

Fox No.	Weight	Number of worms passed		Number of worms found post-mortem		Efficacy in expelling—	
		Hook-worms	Ascarids	Hook-worms	Ascarids	Hook-worms	Ascarids
	Kilos					*Per cent*	*Per cent*
1331	4.0	13	0	0	0	100	
1332	4.0	10	0	0	0	100	
1333	4 7	33	3	0	0	100	100
Total		56	3	0	0	100	100

On all three foxes, records for which are given in Table 3, the tetrachlorethylene produced a laxative effect.

To determine whether the simultaneous administration of 1 grain of phenolphthalein along with tetrachlorethylene at a dose rate of 0.2 c. c. per kilo would affect the efficacy of the anthelmintic, tests were conducted, the results of which are summarized in Table 4.

TABLE. 4.—*Anthelmintic efficiency of tetrachlorethylene given at a dose rate of 0.2 c. c. per kilo together with 1 grain of phenolphthalein*

Fox No.	Weight	Number of worms passed		Number of worms found post-mortem		Efficacy in expelling—	
		Hook-worms	Ascarids	Hook-worms	Ascarids	Hook worms	Ascarids
	Kilos					*Per cent*	*Per cent*
1334	3 1	12	0	0	0	100.0	
1335	3.1	9	0	26	0	25.7	
1336	3.3	1	0	36	0	2.7	
Total		22	0	62	0	26.1	

The phenolphthalein produced a laxative effect only in the case of fox No. 1335; the other two were constipated.

TOXICITY TESTS AND FINDINGS

In order to determine whether foxes are likely to suffer inhalation intoxication when tetrachlorethylene or its fumes are inhaled as a result of the accidental breaking of capsules during administration, six 1 c. c. capsules of the drug were broken in the mouth of each of two foxes. With the exception of a temporary irritation of the mouth, as evidenced by profuse salivation and a rubbing of the mouth with the paws, no apparent ill effects were produced by the drug. Under similar conditions the use of carbon tetrachloride would undoubtedly have brought about collapse and possibly death from inhalation intoxication.

To determine the effects of tetrachlorethylene administered in large doses by means of a stomach tube, the following tests were performed the next day on two foxes:

Fox No. 1349 (total dose, 30 c. c.; dose rate, 8.6 c. c. per kilo).—Vomited food given about 2 hours after dosing; manifested no other apparent symptoms; killed the eighth day after treatment.

Fox No. 1350 (total dose, 30 c. c.; dose rate, 7.5 c. c. per kilo).—Manifested no apparent symptoms; killed the eighth day after treatment.

To determine the microscopic changes produced in the liver by tetrachlorethylene, specimens were selected from the livers of 20 foxes, fixed either in 10 per cent formalin solution or Zenker's fluid, and sent to the Pathological Division of the Bureau of Animal Industry. In all cases the specimens were selected from those portions of the liver where evidences of pathological changes were most apparent macroscopically. Histological sections from these specimens were prepared and examined by Gilbert T. Creech, of the Bureau of Animal Industry. The findings in the livers of these foxes, as reported by Doctor Creech, are as follows:

Fox No. 1327 (dose rate, 0.2 c. c. per kilo).—The liver showed engorgement of the central veins, with distention of adjacent capillaries. The liver cells as a whole presented a pale or granular appearance, and certain of the lobules, particularly those toward the central veins, contained cells in varying stages of degeneration. Many of the cells showed a loss of nuclei, and some had completely atrophied. A few necrotic foci were seen in this specimen, and a few areas of round-cell infiltration were also noted. There was a limited amount of edema present.

Fox No. 1328 (dose rate, 0.2 c. c. per kilo).—The liver showed slight venous and capillary engorgement. The changes were somewhat similar to those noted in the liver of fox No. 1327. Degeneration of the liver cells was evidenced by cloudiness and the loss of nuclei. Vacuolation of the liver cells was quite noticeable in this case.

Fox No. 1329 (dose rate, 0.2 c. c. per kilo).—The liver contained considerable blood, mostly congested in the capillaries. There was marked edema in this case. The liver cells were in stages of degeneration varying from a slight cloudiness of the chromatin to complete atrophy. Vacuolation of the liver cells was also noted.

Fox No. 1332 (dose rate, 1 c. c. per kilo).—The liver was acutely venous and displayed capillary congestion, with small hemorrhages and also a limited amount of edema. Degeneration and atrophy of the cells were noted, particularly in the region of the central veins. Coagulated blood was seen in a number of the vessels, and several small areas of round-cell infiltration were noted.

Foxes Nos. 1334 and 1336 (dose rate, 0.2 c. c. per kilo and 1 grain phenolphthalein).—A number of the liver lobules showed venous and capillary engorgement. Perivascular cellular infiltration was noted in several places. A large percentage of the liver cells showed degenerative changes, and in certain lobules columns of liver cells had undergone complete atrophy. Vacuolation of the liver cells was seen throughout the section. There was rather pronounced edema in this case.

Fox No. 1337 (dose rate, 0.2 c. c. per kilo).—The capillaries toward the center of the liver lobe were engorged with red cells, which stain very poorly. There appeared to have been more or less of a blood stasis in portions of this liver. A number of the larger vessels contained thrombi. A large proportion of the liver cells appeared to be undergoing degenerative changes. In fact there were few normal cells to be seen. An organized exudate was seen surrounding the walls of a number of the vessels.

Foxes Nos. 1338 and 1339 (dose rate, 0.2 c. c. per kilo).—The blood changes in these livers were slight. The specimens showed a noticeably edematous condition. Practically all the liver cells showed cloudiness, and many a loss of nuclei. A number of irregular spaces were seen in which the liver cells had undergone complete atrophy.

Fox No. 1340 (dose rate, 0.3 c. c. per kilo).—The liver showed a passive congestion, and there appeared to have been a blood stasis in the central portion of the lobe. A number of the large vessels showed thrombic formations. The liver cells showed advanced degenerative changes, and in places rather large areas of cell necrosis were seen. In a number of places cellular infiltrations of the interstitial tissue were noted. The capillary engorgement and degenerative process were less marked toward the periphery of the organ.

Fox No. 1341 (dose rate, 0.3 c. c. per kilo).—There had been considerable circulatory disturbance in the liver. The large vessels contained thrombi, and there was capillary engorgement, particularly toward the central portion of the lobe. An organized exudate was seen surrounding many of the larger blood vessels. The degenerative changes in the cells were quite similar to those in the liver of fox No. 1340. A limited amount of perivascular cellular infiltration was seen in this specimen.

Foxes Nos. 1342, 1343, 1344, and 1345 (dose rate, 0.3 c. c. per kilo).—The changes in the livers, consisting of thrombic formations in the larger vessels, capillary blood stasis, and degenerative and atrophic changes in the liver cells, simulated rather closely those described in the livers of foxes Nos. 1340 and 1341.

Fox No. 1346 (dose rate, 0.2 c. c. per kilo).—Circulatory disturbance was noticeable in this case. The larger vessels showed thrombic formations, and the capillaries in the central lobules were distended with degenerated erythrocytes. Edema with coagulated exudate could be seen surrounding many of the larger vessels. There was marked cloudy swelling of the liver cells, the degenerative and atrophic changes being in evidence throughout the sections examined.

Fox No. 1347 (dose rate, 0.2 c. c. per kilo).—The changes were similar to those noted in the liver of fox No. 1346.

Fox No. 1348 (dose rate, 0.2 c. c. per kilo).—The circulatory disturbance and degenerative changes in the liver cells simulated those seen in the livers of foxes Nos. 1346 and 1347. The vacuolation of the liver cells was somewhat more pronounced in this specimen, and a number of small necrotic foci were noted, a few of which showed round-cell invasion.

Fox No. 1349 (dose rate, 8.6 c. c. per kilo).—The capillaries in the central portion of the liver were engorged with degenerated red cells, giving an appearance of blood stasis. Many of the larger vessels contained thrombi. Advanced degenerative changes in the liver cells were seen in this specimen, and areas of cell necrosis were seen throughout the sections examined.

Fox No. 1350 (dose rate, 7.5 c. c. per kilo).—The histological changes in the liver closely simulated those described in connection with the liver of fox No. 1349.

DISCUSSION

The anthelmintic efficiency of the various dose rates of tetrachlorethylene used in the foregoing tests may be summarized as follows:

A 0.2 c. c. per kilo dose removed 85 hookworms from 10 infested foxes and left 2, an efficacy of 97.7 per cent, and removed 15 ascarids from 4 infested foxes and left none, an efficacy of 100 per cent,

A 0.3 c. c. per kilo dose removed 59 hookworms from 5 infested foxes and left none, an efficacy of 100 per cent, and removed 2 ascarids from 2 infested foxes and left none, an efficacy of 100 per cent.

A 1.0 c. c. per kilo dose removed 56 hookworms from 3 infested foxes and left none, an efficacy of 100 per cent.

A 0.2 c. c. per kilo dose, *together with 1 grain of phenolphthalein* removed 22 hookworms from 3 infested foxes and left 62, an efficacy of 26.1 per cent.

In agreement with the findings of Hall and Shillinger (*3*), and also those of Schlingman (*5*), the results of these experiments indicate that tetrachlorethylene without the simultaneous administration of a purgative is highly effective against hookworms and apparently very effective against ascarids.

Since the foregoing experiments on foxes were performed, a paper has reached the author in which Schlingman (*6*) reports tests of tetrachlorethylene on seven foxes and other animals. In his tests the doses of the drug used on foxes ranged from 0.26 c. c. to approximately 3.0 c. c. per kilo. In all cases where the foxes were infested, the drug was found 100 per cent effective against both hookworms and ascarids.

A comparison should be made of the anthelmintic efficacy of tetrachlorethylene and carbon tetrachloride, heretofore the best-known drug for the treatment of foxes for the removal of hookworms. In the foregoing tests tetrachlorethylene alone at the indicated dose rate of 0.2 c. c. per kilo was 97.7 per cent effective against hookworms, removing 85 of the 87 hookworms from 10 infested foxes, and was 100 per cent effective against ascarids, removing all 15 ascarids from 4 infested foxes.

In similar tests of carbon tetrachloride alone at a dose rate of 0.2 c. c. per kilo, Hanson and Van Volkenberg (*4*) found the drug 97.7 per cent effective against hookworms, removing 44 of the 45 hookworms from four infested foxes, and 89 per cent effective against ascarids, removing 24 of the 27 ascarids from three infested foxes.

On the basis of this comparison the two drugs are about equally effective in the removal of hookworms from foxes, and tetrachlorethylene apparently is more effective than carbon tetrachloride in the removal of ascarids. This is in keeping with the conclusions reached by Hall and Shillinger (*3*) on the basis of a similar comparison of tests on dogs.

The simultaneous administration of phenolphthalein caused the efficacy of the indicated dose (0.2 c. c. per kilo) of tetrachlorethylene against hookworms to drop from 97.7 per cent to 26.1 per cent. This result also is in keeping with the findings of Hall and Shillinger (*3*), who found that the effectiveness of tetrachlorethylene was diminished by the simultaneous administration of a purgative.

Tetrachlorethylene, in contrast with carbon tetrachloride, apparently is not attended by danger of inhalation intoxication, as is indicated by the fact that breaking several capsules of the drug in the mouths of two foxes failed to produce symptoms of this type of intoxication. Schlingman (*5*) concluded that the difference in the effects of the two drugs in this respect is due to the fact that tetrachlorethylene is considerably less volatile than carbon tetrachloride.

None of the 26 foxes used in the foregoing tests died from effects of treatment. The only animal (fox No. 1349) to manifest untoward symptoms was one given the enormous dose of 8.6 per kilo. This animal vomited the first meal fed after dosing, but showed no other ill effects. The fact that five of the foxes tolerated doses greatly in excess of the indicated dose of 0.2 c. c. per kilo, these animals

surviving doses ranging from 1 c. c. to 8.6 c. c. per kilo, indicates that the margin of safety for tetrachlorethylene is very high in the absence of contraindications.   This finding is in agreement with the findings of Hall and Shillinger (*3*) and Schlingman (*5*) in tests on dogs and with those of Schlingman (*6*) in tests on foxes.

The gross pathological changes which apparently were due to tetrachlorethylene seemed to be restricted to the liver.   Some of the livers gave evidence of degenerative changes and lobular hemorrhage.   Macroscopically the changes appeared to be irregularly distributed, some portions of the liver being affected more than others.

In spite of the fact that all the 26 foxes used in the experiments survived treatment with tetrachlorethylene and only 1 manifested apparent symptoms (the animal that had had the enormous dose of 43 times the effective dose), microscopic examination of the livers of 20 of the foxes indicated that tetrachlorethylene caused liver injury. The injury occurred even in cases where a dose of 0.2 c. c. per kilo was used.   In some of the livers a necrosis of the cells was noted. The histological findings indicate that it would be advisable to give a thorough purgative about one hour after the administration of tetrachlorethylene.   Such treatment ought to diminish systemic absorption of the anthelmintic and thereby decrease the extent of liver injury.   In cases where the tetrachlorethylene is given in soft elastic globules, it would be advisable to defer the administration of the purgative until about two hours after the globules are given. The findings of Schlingman (*6*) in tests on dogs indicate that there is a loss of anthelmintic efficiency when tetrachlorethylene in soft elastic globules is followed in less than two hours by dry Epsom salts.

The observations made in the foregoing experiments indicate that phenolphthalein in a dose of 1 grain is not sufficiently effective as a purgative to use in conjunction with tetrachlorethylene in the treatment of foxes.   This drug failed to produce a laxative effect in two of the three foxes to which it was given.   Undoubtedly either Epsom salts or castor oil would be more satisfactory than phenolphthalein.

On the basis of the findings of Schlingman (*5*) in tests on dog puppies, it might be inferred that young nursing fox puppies may tolerate treament with the effective doses of tetrachlorethylene.   On this point conclusions should be withheld, however, until sufficient investigation has been carried on to determine the tolerance of nursing fox pups to tetrachlorethylene.

Hall and Shillinger (*3*) state that "the contraindications for tetrachlorethylene will probably be identical with those for carbon tetrachlorid."   Among the probable contraindications they list the following: Febrile and debilitating diseases, extreme youth or old age, gastroenteritis, liver lesions, prolonged fasting, diets of fats, oils, and cream, and simultaneous administration of alcohol.

Davis (*1*), in experiments on dogs, found that factors of diet exerted an enormous influence upon the toxicity of carbon tetrachloride.   It is quite probable that factors of diet will exert a similar influence on the toxic ty of the related compound, tetrachlorethylene. In summarizing his experiments regarding the influence of diet upon the liver injury produced by carbon tetrachloride, Davis states that "Mixed diets and high protein diets—which may be considered normal

for dogs—are more or less protective. High carbohydrate diets afford very striking protection against liver injury. Starvation, on the other hand, is harmful. 'All fat' diets preceding carbon tetrachloride administration are conducive to a maximal injury of liver parenchyma."

CONCLUSIONS

Tetrachlorethylene is as effective as carbon tetrachloride in the removal of hookworms from foxes, and apparently more so in the removal of ascarids. The indicated dose is 0.2 c. c. per kilo, or 1 c. c. per 11 pounds, making 1 c. c. about the proper dose for the average-sized adult fox.

In contrast with carbon tetrachloride, in cases when the containing capsules accidently break, the administration of tetrachlorethylene apparently is not attended with the danger of inhalation intoxication.

The contraindications for tetrachlorethylene probably are the same as those for carbon tetrachloride. In the absence of contraindications, the safety factor for tetrachlorethylene is very high.

Tetrachlorethylene, even in therapeutic doses, may produce liver injury.

The simultaneous administration of a purgative or laxative diminishes the efficacy of tetrachlorethylene. It is advisable to give an effective purgative one to two hours after administration, as so doing probably lessens intestinal absorption of the anthelmintic and diminishes the extent of liver injury.

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EFFECT OF CARBON DIOXIDE UPON THE GERMINA- TION OF CHLAMYDOSPORES OF USTILAGO ZEAE (BECKM.) UNG.[1]

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INTRODUCTION

Much interest has been shown during the past few years in the stimulating effect of various plant tissues upon the germination of spores. Not only has this stimulation occurred when the plant tissue was in contact with the spores or present in the culture medium, but, as has been shown by experiments, some volatile substance from the plant tissue strongly affects spore germination.

Brown (*3*) [2] first demonstrated this stimulating effect upon the germinating spores of *Botrytis cinerea, Botrytis parasitica (allii), Monila fructigena,* and *Penicillium glaucum* by placing them in Petri dishes containing certain plant tissues. He concluded:

The germination of *Botrytis cinerea* spores is increased by the action of volatile substances arising from certain plant tissues, such as apple leaves and fruit, leaves of *Ruta, Eucalyptus,* etc.

He stated further:

It was shown in earlier papers of this series that the conditions present in the infection drop are in general those of feeble nutrition. The vigor of germina- tion in the infection drop—a factor of primary importance in deciding whether attack is possible or not—has been shown in No. VIII of the present series to be influenced by the passive exosmosis of food substances from the host tissue into the infection drop. The present results show that, granted suitable con- ditions, a like effect can be produced through the action of volatile substances arising from the plant and accumulating in the atmosphere.

As is evident from the above quotation, Brown considered that the stimulating effect of the volatile organic substances arising from certain plant tissues was a nutritive effect upon the spores, thereby producing an increased "vigour of germination." It must be re- membered, however, that in determining the amount of germination, he measured a large number of germ tubes and divided the total length of the germ tubes by the number of spores counted. This is a confusion of germination, with growth after germination · Growth after germination is not always an exact index of the amount of ger- mination that has taken place. For instance, 10 germinated spores out of a possible 100 may, by increased growth of their germ tubes, obtain the same average germ-tube length as 20 germinated spores out of a possible 100 under conditions less favorable for growth. In the latter case, however, the percentage of germination would be twice as great as that in the former case.

Noble (*10*), working with *Urocystis tritici* Koern., obtained a stimulatory effect by germinating the spores in the presence of "un-

injured seedlings of nonsusceptible plants (field peas, beans, and rye)." He concluded:

Such stimulation might be expected in the field when the environment causes a certain amount of anaerobic respiration of the plant roots.

He also obtained a stimulatory effect by placing the spores in distilled water in Syracuse dishes and putting them in a vessel containing a small amount of "expressed sap of wheat seedlings." His conclusion as to the stimulating effect in that instance was that—

It is possible in these cases that the stimulus may have been derived from some of the original constituents of the plant sap, or it may have been derived from some of the products of its decomposition, possibly organic acids, esters, or alcohols.

He correlated the stimulating effect obtained in the above experiments with stimulation obtained by the use of such organic substances as benzaldehyde, salicylaldehyde, butyric acid, and acetone. His suggestion regarding the action of the stimuli is "that the action of the stimulatory agents is mainly such as to cause a change in the physical condition of the protoplasmic spore contents, and thus increase the permeability of the latter."

Durrell (6) found that, when certain plant tissues were placed in receptacles containing spores of *Basisporium gallarum* (out of contact with the germination medium), a stimulating effect on germination was produced. This stimulating effect, however, was not obtained when the experiment was conducted in the presence of a barium hydroxide solution. He concluded that the stimulation was due to the carbon dioxide given off by the plant tissues and found that a like stimulation could be obtained by placing the spores in an atmosphere of from 1 to 5 per cent of carbon dioxide.

The above-mentioned investigators, using the spores of various fungi, have definitely shown a stimulatory effect of plant tissue and of carbon dioxide. However, they have not explained the mechanism of this action The work of Brown (2) on the inhibiting effect of carbon dioxide on fungal growth has cast some doubt as to the possibility of any stimulating effect of carbon dioxide when used in such arge quantities as were used by Durrell in his studies of *Basisporium gallarum*. Further research therefore seemed advisable to determine the real effect of plant tissue and carbon dioxide. It is the purpose of the following study, using the spores of *Ustilago zeae*, to show in more detail the effect of plant tissue and carbon dioxide upon spore germination.

EFFECT OF PLANT TISSUE ON THE GERMINATION OF SPORES OF USTILAGO ZEAE

It is not known to what extent a stimulatory effect upon spore germination is a general characteristic of living plant tissue. In order to demonstrate this, tests were made with 20 different plant tissues, a greater variety than was used by the above-mentioned investigators.

In these tests a 3 per cent solution of agar was poured into sterilized Petri dishes. After the agar had cooled, microscope slides on which were placed the solid tissues used, or small vials containing the liquid substances used, were placed in the dishes upon the agar. The spores were then dusted over the remaining surface of the agar and were, therefore, not in contact with the plant tissue (fig. 1). The cultures

were kept at a temperature of 30° C., which had been found by Jones (8) to be optimum for the germination of the spores of *Ustilago zeae*.

In determining the amount of germination, three representative ɩelds in the dishes were selected, each containing from 75 to 100 spores. The average number of germinated spores in the three fields expressed as a percentage of the entire number of spores in the three fields was considered a fair index of the amount of germination in each case.

Table 1 shows the results of 10 trials in which readings on the percentage of germination and the production of sporidia were made at intervals of 3, 6, 12, and 24 hours.
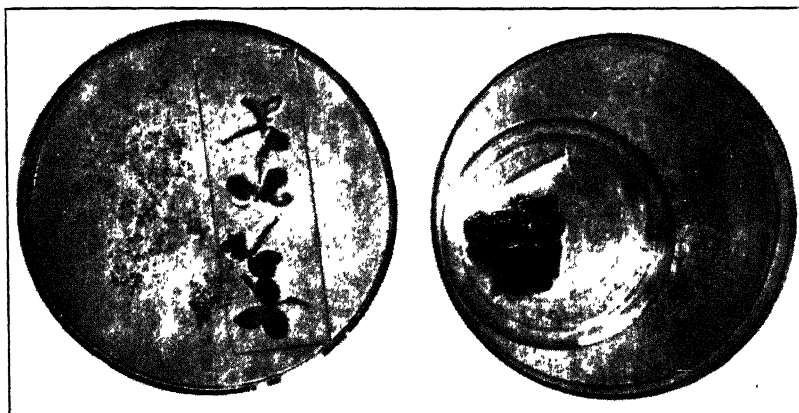
FIG. 1.—Petri dishes containing agar cultures of chlamydospores and plant tissue used in preliminary stimulation studies

TABLE 1.—*Stimulating effects of plant tissue upon the germination of the chlamydospores of Ustilago zeae*

Plant tissue used as stimulant	Results after 3 hours		Results after 6 hours		Results after 12 hours		Results after 24 hours	
	Percentage of germination	Production of sporidia	Percentage of germination	Production of sporidia	Percentage of germination	Production of sporidia	Percentage of germination	Production of sporidia
Control	None	None	Trace.	None	2 6	None	43 1	Fair.
Sweet pea petal	Trace	do	1.4	do	9 2	Trace	60.0	Very abundant.
Poppy petal	do	do	1.8	do	6.8	do	60.3	Do.
Rose petal	do	do	2.2	do	12 5	Fair	84.2	Do
Lilac blossom	do	do	2 2	do	14.2	do	64.5	Abundant.
Spirea blossom	do	do	3.3	Trace	28 3	do	86 5	Very abundant.
Apple leaf	do	do	2.2	None	6.2	Trace	60 8	Abundant.
Geranium leaf	do	do	3.8	Trace	20.0	Fair	85.8	Very abundant.
Corn leaf	do	do	1.4	None	5.6	Trace	56 5	Do.
Rhubarb petiole	do	do	1.6	Trace	12.5	do	80 8	Do.
Pine needles	None	do	1.5	None	6.5	do	62 8	Do
Dill	do	do	Trace	do	6 3	do	59 5	Fair
Green tomato	Trace	do	3.3	Trace	19 2	do	84 3	Very abundant.
Ripe tomato	do	do	3.0	do	28.2	do	86.5	Do.
Apple peel	None	do	Trace.	None	9.5	Trace	80 6	Abundant.
Apple pulp	Trace	do	1.9	do	11.3	do	80.3	Do.
Orange peel	do	do	2 5	do	15.3	do	82 3	Do.
Orange pulp	do	do	2.5	do	12 5	do	81.0	Do.
Lemon peel	do	do	2.5	do	17.2	do	82.3	Do.
Lemon pulp	do	do	1.8	do	15 5	do	72.3	Do.
Strawberry	do	do	1.2	do	7.5	do	69 8	Do.

It may be seen from Table 1 that all the substances used had a stimulating effect upon the germination of the spores. The stimulation is manifest not only in the percentage of germination but also in the character of the germination, a greater production of sporidia being characteristic of the stimulated cultures. Moreover, in the cultures containing plant tissue the spores germinated several hours earlier than in the control cultures. This increase in the speed of germination correlates well with the subsequent production of abundant sporidia. The point of greatest interest, however, is the fact that such a variety of plant tissues stimulate spore germination. This seems to preclude the theory that the stimulation is due to the action of specific volatile substances peculiar to certain plant tissues, and suggests that it is due to some volatile substance universally given off by plant tissues.

RELATION OF CARBON DIOXIDE TO THE STIMULATORY ACTION OF PLANT TISSUE ON SPORE GERMINATION

Tashiro (*12, p. 11*) found that crushed or bruised plant tissue liberates a large amount of carbon dioxide in a very short time. In order to demonstrate more fully that carbon dioxide given off by plant tissue has a stimulatory action on spore germination, the spores of *Ustilago zeae* were dusted on a 3 per cent nonnutient solution of agar in Syracuse dishes which were subsequently placed in air-tight moist chambers. The plant tissues (crushed orange or shredded green corn leaves) were placed in open dishes set in the moist chambers. A saturated solution of barium hydroxide (100 c. c.) was poured into some of the moist chambers to absorb any carbon dioxide that might be given off by the plant tissue.

In another series of experiments, similar moist chambers were exhausted to a partial vacuum and allowed to fill with air that had passed through a flask containing the plant tissue and then through three wash bottles of the barium hydroxide solution. A subsequent constant flow of air from the plant-tissue chamber through the barium hydroxide and into the germination chambers was maintained by an aspirator. A parallel series of experiments was conducted, which was similar to the above in every detail except that the spores were dusted into dry, clean Syracuse dishes instead of onto moist agar.

As controls in the above experiments, some moist chambers were used which contained only water to produce a humid atmosphere and some which contained only the plant tissue. The cultures were kept at a temperature of 30° C., and the germination counts were made after 24 hours. A summary of the results obtained in 10 trials is given in Table 2.

It will be noted in Table 2 that in the control cultures of agar containing no plant tissue a germination of 42.8 per cent was obtained. Cultures containing 75 to 100 grams of crushed orange germinated 91.8 per cent, while similar cultures in the presence of 100 to 150 grams of shredded corn leaves germinated 83.2 per cent.

In the presence of barium hydroxide a germination comparable to the control cultures containing no plant tissue was obtained. The control cultures on dry glass surfaces containing no plant tissue showed a trace of germination. When plant tissue was placed in the chambers containing the cultures on dry glass surfaces about 10 per

_cent germination resulted. Here again, in the presence of barium hydroxide, a germination comparable to the control cultures containing no plant tissue was obtained. The water of condensation collecting on the spores dusted on dry glass surfaces seemed sufficient to produce the germination of some of the spores. This water could be readily seen through the microscope. In general, the results shown in Table 2 indicate that the stimulatory agent affecting spore germination in the presence of plant tissue is carbon dioxide.

TABLE 2.—*Inhibitory effect of barium hydroxide upon the stimulation of spore germination by plant tissue*

Medium	Modification of environment	Percentage of germination
Agar	Control (no plant tissue)	42.8
Do	Control (75 gm. to 100 gm of crushed orange)	91.8
Do	Control (100 gm to 150 gm. of shredded corn leaves)	83.2
Do	Crushed orange and barium hydroxide	51.7
Do	Shredded corn leaves and barium hydroxide	48.3
Do	Air passed over crushed orange and through barium hydroxide	44.3
Do	Air passed over shredded corn leaves and through barium hydroxide	39.6
Glass surface	Control (no plant tissue)	Trace.
Do	Control (75 gm. to 100 gm. of crushed orange)	11.4
Do	Control (100 gm. to 150 gm. shredded corn leaves)	9.8
Do	Crushed orange and barium hydroxide	6.1
Do	Shredded corn leaves and barium hydroxide	4.2
Do	Air passed over crushed orange and through barium hydroxide	Trace.
Do	Air passed over shredded corn leaves and through barium hydroxide	Trace.

QUANTITY OF CARBON DIOXIDE PRODUCED BY PLANT TISSUE

Durrell (*6*) in his work gave little consideration to the quantity of carbon dioxide given off by the plant tissues used. A series of experiments was therefore conducted by the present writers to determine the quantity of carbon dioxide produced by plant tissue in amounts comparable to those used in the above germination tests. It has been previously noted that from 75 to 100 grams of crushed orange and from 100 to 150 grams of shredded corn leaves produced the best stimulatory effect in moist chambers with a capacity of 2 liters. The quantity of carbon dioxide given off by these tissues was determined by passing air free from carbon dioxide into chambers containing the tissues and then into a saturated solution of barium hydroxide. A constant flow of air through the apparatus, maintained by means of an aspirator, produced a heavy precipitate of barium carbonate, which was measured by titration, as described by Harter and Weimer (*7*). In these experiments atmospheres containing from 10 to 15 per cent of carbon dioxide were obtained in the culture chambers.

ACIDITY IN SPORE-CULTURE MEDIA PRODUCED BY CARBON DIOXIDE FROM PLANT TISSUE

In view of the fact that water absorbs carbon dioxide very readily, thereby increasing the acidity of the solvent, and that acidity markedly affects cell permeability, it seemed logical to analyze the stimulatory effect of carbon dioxide upon spore germination by determining the change of hydrogen-ion concentration produced in the culture media by the presence of plant tissue.

The apparatus used in this study is shown in Figure 2. Drops of media containing suitable indicators were suspended in small vials containing bits of plant tissue such as tomato pulp, orange pulp, apple pulp, green corn leaves, wheat seedlings, and corn seedlings. As controls, drops were suspended in other vials each containing a small amount of barium hydroxide solution. The drops were suspended by drawing the liquid media containing the indicators into glass pipettes inserted through the corks of the vials. The tops of the pipettes were plugged with plasticene. Pressure on the soft plasticene plugs caused the colored media to exude in drops from the
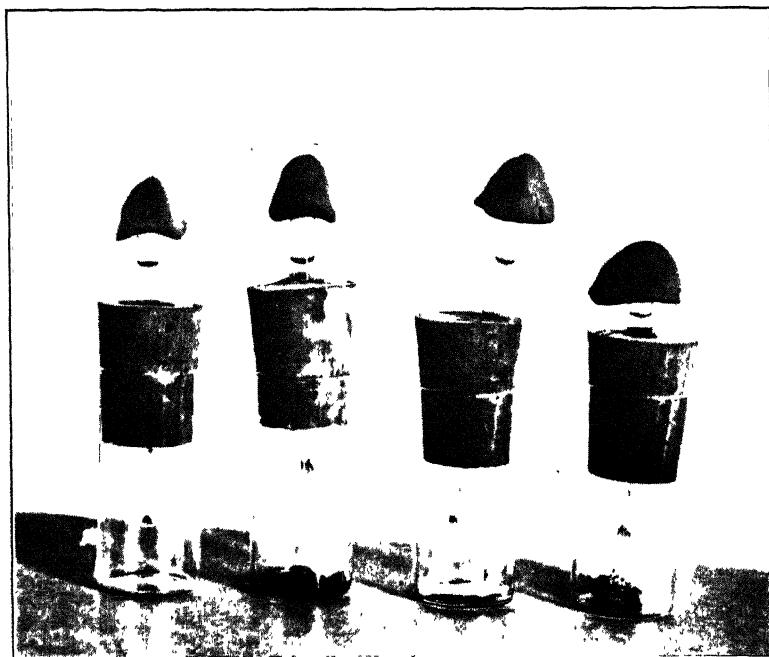
FIG. 2.—Drops (containing indicators) suspended in chambers above fragments of plant tissue

end of the pipettes within the vials. Conditions herein described are comparable to hanging-drop cultures in Van Tieghem cells. The vials were kept at a constant temperature for 24 hours and were then compared with standard indicators (5). The results of this experiment are shown in Table 3.

TABLE 3.—*Effect of the presence of plant tissue upon the hydrogen-ion concentration of media*

Medium	Atmospheric conditions	P_H at beginning of experiment	P_H at end of experiment
Tap water	Control (air over Ba(OH)₂)	6.7	6.7
Do	Control (air only)	6.7	5.5
Do	Tomato tissue present	6.7	4.9
Do	Orange tissue present	6.7	4.9
Do	Apple tissue present	6.7	4.9
Do	Wheat seedlings present	6.7	4.9
Do	Corn seedlings present	6.7	4.9

As is evident from Table 3, carbon dioxide given off by the plant tissue changed the acidity of the media in the drops from 6.7 P_H to approximately 4.9 P_H. It was difficult to make these determinations accurately, since refraction of light affected the color of the drops. The results, however, plainly indicate that the amount of carbon dioxide given off by plant tissue and absorbed by the drops was sufficient to change the acid reaction.

In order to obtain more accurate data, 5 c. c. of the medium (tap water, a 3 per cent agar solution or a 10 per cent gelatin solution) containing suitable indicators were poured into each of several 125 c. c. bottles of the same interior diameter as the Syracuse dishes used in the germination studies. The air was first exhausted from these bottles by means of a vacuum pump. They were then filled with carbon dioxide and air in varying proportions. Atmospheres containing 5, 10, 15, 20, 25, and 30 per cent of carbon dioxide were used. The bottles were kept at a temperature of 30° C. for 24 hours (conditions maintained in the germination tests). Results of 10 trials in the above experimental work are summarized in Table 4.

TABLE 4.—*The effect of CO_2 upon the P_H of media*

Medium	Percentage of CO_2 used	P_H at beginning of experiment	P_H at end of experiment
Tap water	5	6.5	5.40
Do	10	6.5	5.20
Do	15	6.5	4.95
Do	20	6.5	4.85
Do	25	6.5	4.75
Do	30	6.5	4.65
3 per cent agar	5	6.5	6.40
Do	10	6.5	5.80
Do	15	6.5	5.83
Do	20	6.5	5.10
Do	25	6.5	4.95
Do	30	6.5	4.85
10 per cent gelatin	5	6.7	6.30
Do	10	6.7	5.90
Do	15	6.7	5.65
Do	20	6.7	5.25
Do	25	6.7	5.10
Do	30	6.7	4.95

In Table 4 it may be noted that the hydrogen-ion concentration of the media is appreciably changed by atmospheres containing from 5 to 30 per cent of carbon dioxide. Atmospheres containing 15 per cent of carbon dioxide, such as had been found favorable for the germination of the spores of *Ustilago zeae* in the presence of plant tissue, changed the hydrogen-ion concentration of tap water from 6.5 to 4.9. The acidity change of the colloidal substances was not as great. The P_H of agar was changed from 6.5 to 5.3 and of gelatin from 6.7 to 5.6. This is in accordance with the findings of Cornelia Lee Carey (4), who showed that the intake of carbon dioxide in colloidal substances is proportional to the water content, the water acting as a medium for the absorption of this gas.

THE RELATION OF THE ACIDITY CHANGE PRODUCED BY CARBON DIOXIDE TO SPORE GERMINATION

To further correlate the above findings with the results of germination studies, *Ustilago zeae* spores were germinated in atmospheres containing various quantities of carbon dioxide. In these tests, the results of which are tabulated in Table 5, the air was exhausted from the culture chambers and replaced with atmospheres containing the following percentages of carbon dioxide: 5, 10, 15, 20, 25, 30, 50, and 75. Within the chambers were placed Syracuse dishes containing the media on which the spores were dusted. In order to eliminate stimulation from any foreign substances in the media used, nonnutrient media were chosen. Tap water, a 3 per cent solution of washed agar, a 10 per cent solution of gelatin, a silica jelly, made as Smith (*11, p. 36*) directs, and collodion films, made as Walpole (*13*) suggests, were used. The culture chambers were kept at a temperature of 30° C., and germination counts, previously described, were made after 24 hours.

TABLE 5.—*Stimulating effects of CO_2 upon the germination of the chlamydospores of Ustilago zeae*

Medium	Percentage of CO_2 used	P_H at beginning of experiment	P_H at end of experiment	Percentage of germination (after 24 hours)
Tap water	Control.	6.5	6.50	0 0
Do	5	6.5	5.40	Trace.
Do	10	6.5	5.20	2 0
Do	15	6.5	4.95	6 0
Do	20	6 5	4.85	2.0
Do	25	6.5	4 75	Trace.
Do	30	6.5	4.65	Trace.
Do	50	6.5	4.40	0.0
Do	75	6.5	4.25	0 0
3 per cent agar	Control.	6.5	6.50	Trace.
Do	5	6.5	6 40	46.4
Do	10	6 5	5.80	74.1
Do	15	6 5	5 30	88.2
Do	20	6 5	5.10	72.4
Do	25	6 5	4.95	39 6
Do	30	6.5	4.85	21.8
Do	50	6 5	4.55	Trace.
Do	75	6.5	4 30	0.0
10 per cent gelatin	Control.	6 7	6.70	Trace.
Do	5	6 7	6 30	39.2
Do	10	6.7	5.90	53 4
Do	15	6.7	5.65	68.8
Do	20	6.7	5.25	54.6
Do	25	6.7	5 10	43.2
Do	30	6.7	4 95	24.1
Do	50	6 7	4.65	Trace.
Do	75	6.7	4.50	0.0
Silicate gel	Control.			0.0
Do	5			4.4
Do	10			12.6
Do	15			28.2
Do	20			14 2
Do	25			3.8
Do	30			Trace.
Do	50			0.0
Do	75			0.0
Collodion	Control.			[a] Trace.
Do	5			[a] 8.4
Do	10			[a] 74.6
Do	15			[a] 93.2
Do	20			[a] 45 6
Do	25			[a] 9.6
Do	30			[a] Trace.
Do	50			[a] Trace.
Do	75			[a] 0.0

[a] After 48 hours.

The results of 10 trials, as summarized in Table 5, show the germina-
tion in different media in the presence of various percentages of carbon
dioxide.   Table 5 also shows the acidity of the media at the beginning
of the experiments and at the end of the germination period.   The
highest percentage of germination in each medium occurred in an
atmosphere containing 15 per cent of carbon dioxide.   Atmospheres
containing this amount of carbon dioxide changed the hydrogen-ion
concentration of the tap water from 6.5 to 4.9; that of agar from 6.5
to 5.3, and that of gelatin from 6.7 to 5.6.   Kendall (*9*) showed that
under ordinary conditions the partial pressure of carbon dioxide in
the air produces a hydrogen-ion concentration in pure water of 5.69
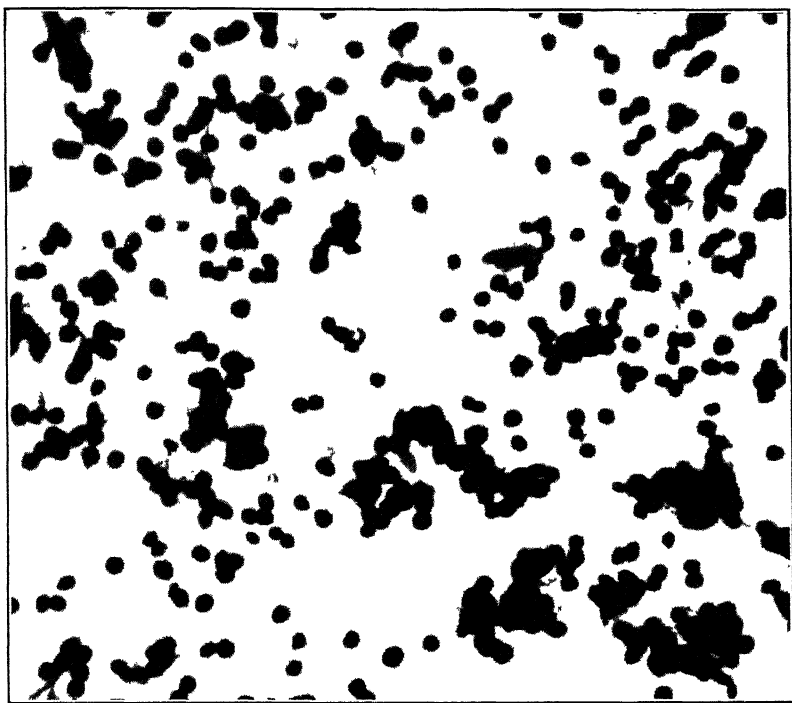
FIG. 3.—Germination of chlamydospores of *Ustilago zeae* on collodion in saturated atmosphere

and that atmospheres containing 100 per cent of carbon dioxide over
water produce an acidity of P_H 3.9.   Somewhat similar data are
presented in Table 5, in which it is shown that atmospheres contain-
ing higher concentrations of carbon dioxide produced greater acidity
of the media.

The optimum acidity for the germination of the spores, as obtained
with carbon dioxide, is probably about P_H 4.9.   This is the hydrogen-
ion concentration produced in tap water subjected to atmospheres
containing 15 per cent of carbon dioxide.   Its optimum relation to
the germination of the spores was observed in the water cultures
and in the cultures in which the spores were dusted on collodion
films or glass surfaces.   In the use of collodion or glass surfaces as

substrata, the water of condensation was the only source of moisture and was sufficient for germination. In the case of the colloidal media (agar and gelatin), sufficient carbon dioxide to change the acidity of the entire mass to the optimum P_H changed the acidity of the surface film of water, the true medium of the germinating spores, to a point below the optimum. That this was the case was brought out in connection with the experiments in which bottles containing agar or gelatin were filled with mixtures of carbon dioxide and air, previously described. Furthermore, in atmospheres containing 15 per cent of carbon dioxide the surface film of water reached the optimum acidity long before an equilibrium was established throughout the entire medium.

It is interesting to note in Table 5 that excellent germination was obtained when silica jelly and collodion were used as substrata (fig. 3). Since De Bary (*1, p. 349–352*) made the statement that the nutrients in the media in which fungus spores are germinated play a part in their germination, that explanation has been almost universally accepted. The results obtained in the experiments presented in this paper by germinating the chlamydospores of *Ustilago zeae* on silica jelly or collodion do not confirm this theory. These media did not contain any nutrient material, and germination was therefore dependent upon temperature, moisture, carbon dioxide, and oxygen.

It appears from results obtained in these experiments that atmospheres containing 15 per cent of carbon dioxide produce in the culture media a hydrogen-ion concentration of 4.9, which is optimum to the germination of the chlamydospores of *Ustilago zeae*.

SUMMARY

Experiments on the stimulatory effect of the presence of living plant tissue upon the germination of the chlamydospores of *Ustilago zeae* (Beekm.) Ung. indicate that a great variety of plant tissues affect the germination of the spores.

Analysis of the stimulation caused by the presence of plant tissue shows that the stimulatory agent is carbon dioxide.

Atmospheres containing 15 per cent of carbon dioxide produced by the presence of plant tissue or by a gas generator were found to be optimum to the germination of the spores of *Ustilago zeae*.

A retarding effect upon the germination of these spores was obtained when atmospheres containing more than 15 per cent of carbon dioxide were used.

The stimulatory effect of carbon dioxide upon the germination of the spores of *Ustilago zeae* is apparently due to a definite action of carbonic acid.

Atmospheres containing 15 per cent of carbon dioxide produced hydrogen-ion concentrations in the spore culture media varying from 4.9 to 5.6, according to the medium used. The optimum acidity for the germination of the chlamydospores of *Ustilago zeae* obtained with carbonic acid is, however, probably nearer P_H 4.9 than P_H 5.6.

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NOTES ON THE LIFE HISTORY AND CONTROL OF THE STRAWBERRY LEAF ROLLER [1]

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INTRODUCTION

Life-history and control studies of the strawberry leaf roller (*Ancylis comptana* Fröhl.) were begun at Ames, Iowa, on June 15, 1923, and were carried on throughout the summer season. When the work was undertaken the larvae of the first generation were numerous, and some had pupated in the strawberry fields in the vicinity of Ames. By June 20 many moths were flying, and a great number of these were collected and placed on strawberry plants which had been potted and covered with 8-inch lantern-globe cages. The adults were kept confined in the cheesecloth-covered chimneys for oviposition records. Sweetened water, sprinkled on the leaves or on pieces of blotting paper, served as food.

LONGEVITY OF ADULTS

The average length of life of the 30 females under observation was found to be 14.73 days, the maximum 34 and the minimum 5 days. The average longevity of 25 males under observation was 16.56 days, the maximum 27 and the minimum 9 days. On June 27 a dozen moths were taken from a breeding cage and held in captivity without food. They lived from 5 to 16 days; on an average, 8.16 days. Detailed longevity data are given below.

Number of days adults lived	Males	Females	Number of days adults lived	Males	Females
5		1	17	2	
8		4	18	5	2
9	1	1	19	2	1
10	2	1	21	1	3
11	1	2	22	1	2
12	1	5	24	1	
13	3		25	1	
14	3	1	27	1	
15		2	34		1
16		4			

Journal of Agricultural Research,
Washington, D C.

Vol 34, No. 2
Jan. 15, 1927
Key No. Iowa-12

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OVIPOSITION

The preoviposition period ranged from 1 to 4 days, averaging 2.3 days. Fertilized females laid eggs quite freely while in captivity. Many newly emerged unfertilized females were placed in cages on strawberry plants, and at the end of the fifth day some had deposited infertile eggs and some showed no signs of oviposition. In all of the breeding work a newly emerged male and female were placed in a cage and kept together throughout the period of experimentation. In some cases, however, a male was used to fertilize a second female.

The oviposition period was determined by making daily egg counts and moving the pair of moths to a new cage containing an uninfested plant. The length of the oviposition period of 30 females follows. The oviposition period of the 30 females averaged 9.5 days, the minimum being 4 and the maximum 19 days. The postoviposition period ranged from 1 to 21 days, the average being 3.3 days.

Number of females	Number of days in oviposition period	Number of females	Number of days in oviposition period
1	4	3	11
1	5	2	12
6	6	2	14
4	7	1	15
5	8	1	16
1	9	1	18
1	10	1	19

The number of eggs laid by the 30 females was as follows:

Number of females	Number of eggs laid	Number of females	Number of eggs laid
1	14	1	89
1	33	1	90
2	35	1	93
1	48	2	94
1	52	3	96
1	55	1	99
1	56	1	109
1	61	1	110
1	62	1	122
1	68	1	127
1	71	1	136
1	83	1	161
1	86	1	182

The number of eggs laid per day ranged from 1 to 67 and averaged 9.25. These moths deposited 78.26 per cent of their eggs during the first half of their lives. The average number of eggs laid by one individual was 85.1, the maximum was 182, and the minimum was 14.

INCUBATION PERIOD

The length of the incubation period of the strawberry leaf roller was determined from data taken from 919 hatchings under an outside shelter. Under these conditions when the temperature ranged from 50° to 100° F., the larvae issued 5 to 13 days after deposition, the average interval being 7.9 days (fig. 1). The following
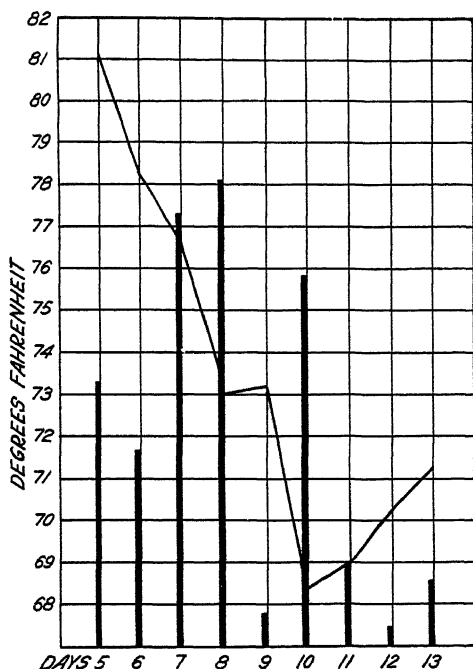
FIG. 1.—Relation of mean temperature to length of incubation period of the strawberry leaf roller. (The length of the bars corresponds to the number of individuals for which records were obtained, the distance from the base to the next horizontal line representing 20 individuals)

tabulation shows that 5 days is the length of the incubation period at 81.1° and that an incubation period of 8 days at 73.02° seems to be the optimum for development.

Number of individuals	Average temperature, °F.	Number of days in incubation period	Number of individuals	Average temperature, °F	Number of days in incubation period
126	81. 1	5	176	68. 4	10
93	78. 2	6	41	69	11
206	76. 57	7	9	70. 3	12
222	73. 02	8	31	71. 3	13
15	73. 2	9			

HABITS OF LARVAE AND INJURY TO PLANTS

The little caterpillars usually feed on the dorsal surface of the leaf, but they may also be found feeding on the under side. The actual damage is caused by their feeding on the leaf tissue. The whole leaf is not eaten, but as a rule it is folded over at the midrib and held in this position by fine silken threads. In this fold the larva feeds on the epidermis, causing the rest of the tissues of this area to turn brown and die, thus making the strawberry foliage look as if it had been scorched by fire. Where the insects are abundant, the leaves, especially those near the bud, are often tied in an indiscriminate fashion.

LENGTH OF LARVAL PERIOD

Data showing the length of the larval period at Ames in 1923, are as follows:

The minimum period for larval development was 16 days at a mean temperature of 79.8°. The maximum period of the larval stages for
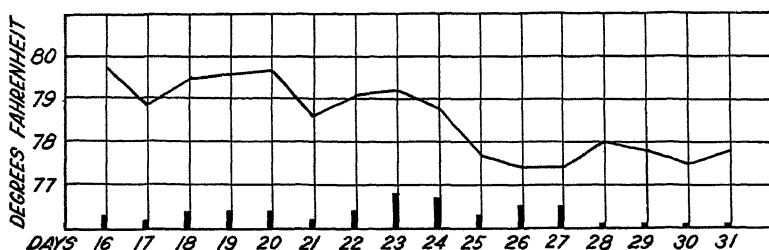
Fig. 2.—Relation of mean temperature to length of larval period of the strawberry leaf roller. (The length of the bars corresponds to the number of individuals for which records were obtained, the distance from the base to the next horizontal line representing 10 individuals)

the summer broods increased to 31 days with only a slight decrease in temperature (fig. 2). The average number of days required for development, based on all the records was 22.7.

Number of individuals	Average temperature, °F.	Number of days in larval stage	Number of individuals	Average temperature, °F.	Number of days in larval stage
3	79. 8	16	7	78. 8	24
2	78. 9	17	3	77. 7	25
4	79. 5	18	5	77. 4	26
4	79. 6	19	5	77. 4	27
4	79. 7	20	1	78	28
2	78. 6	21	1	77. 8	29
4	79. 1	22	1	77. 5	30
8	79. 2	23	1	77. 8	31

LENGTH OF PUPAL PERIOD

The length of the pupal period ranged from 6 to 15 days and averaged 9.6 days. Temperature seemed to have a direct bearing on the number of days required for development. Nevertheless, indi-
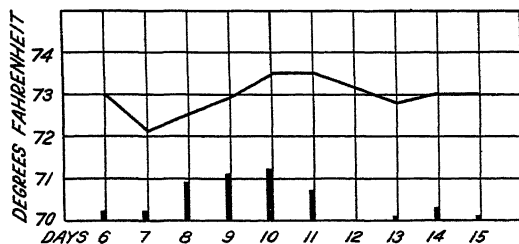
FIG 3.—Relation of mean temperature to length of pupal period of the strawberry leaf roller (The length of the bars corresponds to the number of individuals for which records were obtained, the distance from the base to the next horizontal line representing 10 individuals)

viduals that spent 6 and 15 days, respectively, in the pupal stage developed at a mean temperature of 73° F Other notable exceptions were individuals taking from 7 to 14 days to develop at temperatures ranging from 72.1° to 73.5°. The optimum for development seems to be 10 days at a mean temperature of 73.5° (fig. 3). Detailed records showing length of the pupal period at different temperatures are as follows:

Number of individuals	Average temperature, ° F.	Number of days in pupal stage	Number of individuals	Average temperature, ° F.	Number of days in pupal stage
2	73	6	7	73. 5	11
2	72. 1	7	1	72. 8	13
9	72. 5	8	3	73	14
11	72. 9	9	1	73	15
12	73. 5	10			

TOTAL LIFE CYCLE

Summing up the averages of the number of days required for the development of the different stages, Table 1, gives an average total life cycle of 42.5 days.

TABLE 1.—*Average length of life cycle*

	Number of records	Maximum	Minimum	Average
Number of days of incubation of eggs	919	13	5	7. 9
Larval period (days)	55	31	16	22. 7
Pupal period (days)	48	15	6	9. 6
Preoviposition period (days)	30	4	1	2. 3
Total number of days				42. 5

SEASONAL HISTORY

In 1923 at Ames there were three complete generations of the strawberry leaf roller. Observations in Iowa from 1915 to 1918 definitely established the fact that the leaf roller spends the winter in the strawberry beds as a nearly full-grown caterpillar. Early in the spring when the food supply is available the caterpillars feed to some extent and soon transform to the pupal stage, and a few days later the adult moths appear. These moths of the overwintered brood lay eggs and give rise to the first generation, which appears in late May and early June.[3] By June 15, when this investigation was begun, larvae of all sizes and some pupae were found in the leaves.

By June 29, most of the larvae of this first generation had pupated, and many moths were flying in the fields. By July 3 there were scarcely any larvae and but few pupae of this group to be found, and on July 10 small larvae of the second generation were very numerous in the fields. On August 2 a few moths were emerging, and many full-grown larvae were observed, some of which were pupating. By the 10th of August the moths were emerging freely. The eggs which they deposited gave rise to the third generation. The larvae of this third generation, which fed actively until the strawberry leaves were no longer suitable for food, prepared for themselves quarters for hibernation in the strawberry foliage in the beds.

There was an overlapping of these generations, owing to the great variation in the length of the larval period and some variation in the length of the other developmental stages.

NATURAL ENEMIES

During the latter part of June many spiders were found in the strawberry fields, and most of them were on the rolled leaves. It is thought that they were feeding on the larvae of the leaf roller.

Another important factor in the natural control of these rollers is certain parasitic hymenopterous insects which frequently parasitize the larvae or pupae. These parasites were not found in great numbers, but certain species occasionally became sufficiently abundant to check rapid multiplication of the leaf rollers.

During the summer of 1923 at Ames the writer bred the following parasites from parasitized larvae of *Ancylis comptana: Sympiesis ancylae* Girault, *Meteorus trachynotus* Vier, *Hoplocryptus incertulus* (D. T.), *Spilochalcis albifrons* Walsh, and *Spilocryptus polychrosidis* Cush.[4] The pupal stage of these parasites lasted from five to nine days when they were placed in the cool part of the insectary. All specimens were reared in salve boxes on damp sand.

CONTROL EXPERIMENTS

When the rollers become very abundant control measures must be instituted if a full crop of strawberries is to be harvested. In spite of some natural enemies these insects often breed in great numbers and almost defoliate the plants.

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[3] WEBSTER, R. L. THE STRAWBERRY LEAF ROLLER (ANCYLIS COMPTANA FRÖHL). Iowa Agr. Expt. Sta. Bul. 179 (abridged), 4 p , illus. 1918
[4] The determinations were made by R. A. Cushman and A. B. Gahan, of the Bureau of Entomology, U. S. Department of Agriculture.

The spraying and dusting experiments described in this paper were conducted on an acre of strawberries made available by the horticultural department of Iowa State College. The spraying and dusting were begun June 27, 1923, just as the moths were beginning to oviposit for the second generation. Inasmuch as some of the poison was washed off by a heavy rain late in the afternoon, the spraying and dusting were repeated on July 5. The spray and dust were next applied on July 29, when the moths were beginning to oviposit for the third generation. These tests were made on moderately infested plants which had been set out in the fall of 1922. The rows were 3 feet apart in the field and ran north and south. The plants were spaced about 18 inches in the drill throughout the field. Along the east side of the field were three strawberry patches, one of which was several years old and badly infested. The second and third patches had been set out about two years before the time of this investigation and were less heavily infested. These small adjoining patches covering altogether about one-fourth of an acre, were used as far as possible as a control.

Since it is almost impossible to kill the larvae and pupae in the rolled leaves, the applications of arsenicals were timed to poison the young larvae as they issued from the eggs and started to feed. The following insecticides were used:

Dusts:
    Lead arsenate, 1 pound to 5 pounds of gypsum.
    Calcium arsenate, 1 pound to 10 pounds of gypsum.
    Lead arsenate, 1 pound to 5 pounds of slaked lime.
    Calcium arsenate, 1 pound to 10 pounds of slaked lime.
Sprays:
    Lead arsenate, 1 pound to 50 gallons of water.
    Lead arsenate, 1½ pounds to 50 gallons of water.
    Lead arsenate, 2 pounds to 50 gallons of water.
    Lead arsenate, 1 pound to 50 gallons of water, with 1½ pounds of laundry soap.
    Lead arsenate, 1½ pounds to 50 gallons of water, with 1½ pounds of laundry soap.
    Lead arsenate, 2 pounds to 50 gallons of water, with 1½ pounds of laundry soap.

The dusts were applied with a hand duster at the rate of about 25 pounds to the acre; the sprays were applied with a wheelbarrow sprayer at the rate of about 100 gallons to the acre.

After the first application no detailed counts were made to determine the exact effect of the poison on the larvae, but it was easy to see that the larvae were much less numerous on the treated plots than on the controls. On July 29, the second application was made, and on August 3 every plant in the field was thoroughly examined to determine the effectiveness of the different arsenicals. Table 2 shows the results obtained. From this table it is apparent that at the conclusion of the experiments the insects were under almost complete control in all the plots in spite of the fact that they are especially fond of new plants. Only 357 rollers, too few to cause any appreciable damage, were found in the entire acre. The most effective of the arsenicals used was lead arsenate applied at the rate of 1 pound to 5 pounds of gypsum. Lead arsenate at the rate of 1 pound to 5 pounds of slaked lime and calcium arsenate at the rate of 1 pound to 10 pounds of gypsum gave nearly as good results.

The dust consisting of 1 pound of calcium arsenate and 10 pounds of slaked lime was about as effective as the various sprays. Since such small numbers of rollers were dealt with it is almost impossible to interpret the data correctly, but it is seen that where the two sprays were applied which contained only 1 pound of the arsenical to 50 gallons of water the infestation was greater than where sprays were used that contained more of the insecticide.

TABLE 2.—*Comparative effectiveness of the different poisons used against the strawberry leaf roller at Ames, Iowa, in 1923*

Plot No. [a]	Poison used	Number of rollers found after arsenical was applied
1	Lead arsenate, 1 pound to 5 pounds of gypsum (dust)	6
2	Calcium arsenate, 1 pound to 10 pounds of gypsum (dust)	7
3	Lead arsenate, 1 pound to 5 pounds of slaked lime (dust)	7
4	Calcium arsenate, 1 pound to 10 pounds of slaked lime (dust)	42
5	Lead arsenate, 1 pound to 50 gallons of water (spray)	63
6	Lead arsenate, 1½ pounds to 50 gallons of water (spray)	45
7	Lead arsenate, 2 pounds to 50 gallons of water (spray)	45
8	Lead arsenate, 1 pound to 50 gallons of water and 1½ pounds of laundry soap (spray)	59
9	Lead arsenate, 1½ pounds to 50 gallons of water and 1½ pounds of laundry soap (spray)	46
10	Lead arsenate, 2 pounds to 50 gallons of water and 1½ pounds of laundry soap (spray)	36
	Total	357

[a] The area of each plot was one-tenth of an acre.

Three of the dusted areas showed considerably less infestation than the sprayed plots, in spite of the fact that plots 1, 2, and 3 were nearer the old strawberry beds than were the dusted areas. Plot 1 was nearer the old beds than was plot 2, and plot 2 was nearer than plot 3. Almost all the plants in the patches used as controls were completely defoliated when these counts were made.

A STUDY OF DARK, HARD KERNEL AND PROTEIN CONTENT OF HARD RED SPRING WHEAT[1]

By C. E. MANGELS

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INTRODUCTION

Market grades for hard-wheat types have usually provided a classification on the basis of dark or hard-kernel content. For a number of years before the present Federal grades were promulgated the dark, hard-kernel content was used as a basis for grading spring wheat. The present Federal grades provide for three subclasses of hard red spring wheat, as follows: (1) Dark northern spring, containing 75 per cent or more of dark, hard, and vitreous kernels; (2) northern spring, containing less than 75 per cent and more than 25 per cent of dark, hard, and vitreous kernels; and (3) red spring, containing not more than 25 per cent of dark, hard, and vitreous kernels. Hard spring, containing 85 per cent or more of dark, hard, and vitreous kernels, is a premium grade under the subclass dark northern spring.

The present Federal grades provide similar subclasses for hard red winter and durum wheats. The use of the dark or hard-kernel content as a basis for grading has been confined to "hard" wheat types, which will generally average higher in protein or gluten content than other types, and which will also show greater variation in physical characteristics and composition according to season and location. Dark or hard-kernel content is used as a basis for grading hard wheats on the assumption that the percentage of dark, hard, vitreous kernels is an indication of the protein content of the wheat.

It is generally conceded that dark, hard, vitreous kernels contain more protein than light-colored, starchy kernels from the same lot of wheat. Snyder[2] found that "when the two types of seed, light and dark, were selected from the same lot of wheat, the darker seeds in all samples analyzed were found to be richer in protein." Snyder also found that light seeds and dark seeds from different sources contained different amounts of protein.

Roberts[3] attempted to correlate crushing strength of hard winter wheat with protein content, but found no significant correlation. The results of his work indicate that crushing or breaking strength is probably of less importance as indicating protein content than the color of the kernel.

Mangels and Sanderson[4] found a positive correlation between protein content and dark, hard kernels for the crops of 1922, 1923,

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and 1924, but the coefficient of correlation showed considerable seasonal variation.

Under the Federal system of grading hard red spring and hard red winter wheat, the term "dark, hard, and vitreous" kernels is used, but in actual practice wheats are divided into subclasses on the basis of color only. Kernels which are dark in color, however, are usually hard and vitreous, and at present no convenient or practical method of measuring "hardness" or "vitreousness" is available.

The North Dakota Experiment Station has collected data on dark kernels and protein content for four crop years, and now has information on more than 1,000 samples from the crops of 1922 to 1925, inclusive. Because of the importance of the relationship between dark kernels and protein content it was deemed advisable to make a careful study of these data.

CORRELATION BETWEEN PROTEIN CONTENT AND PERCENTAGE OF DARK KERNELS IN HARD RED SPRING WHEAT

The results of correlation studies made on the North Dakota crops of 1922 to 1924 have already been published.[4] Table 1 brings together the results of correlation studies for the four crop years, 1922 to 1925, inclusive.

TABLE 1.—*Correlation between protein content and dark-kernel content of hard red spring wheat from North Dakota crops, 1922–1925*

Year	Number of samples	Mean protein content	Mean dark-kernel content	Coefficient of correlation	Probable error
		Per cent	*Per cent*		
1922	90	12.12	83	0.660	±0.041
1923	199	13.35	73	.067	±.047
1924	316	11.35	79	.453	±.030
1925	436	12.31	90	.299	±.043

The coefficients of correlation for the four crop years show a wide range in magnitude. In the 1922 crop there is a marked correlation between dark kernels and protein content, and in the 1924 crop there is a significant correlation, but in the 1925 crop the correlation is less than 0.3, and in 1923 there is practically no correlation. The 1923 crop also shows the highest mean protein content and the lowest mean dark kernel content, but this apparent discrepancy is due to the fact that many of the samples from the 1923 crop were weather damaged. These weather-damaged samples while high in protein content would on analysis show a large percentage of light-colored or starchy kernels.

A study of frequency diagrams indicates that wheat having a high percentage of dark kernels will show considerable variation in protein content. The frequency diagram for the 1925 crop shows that wheat containing 95 to 100 per cent of dark kernels varied from 10 to 19 per cent in protein content. The coefficient of correlation of the 1922 crop is high because of the fact that samples from this crop containing a high percentage of dark kernels show relatively less spread in protein content than other crops.

⁴ MANGELS, C. E., and SANDERSON, T. THE CORRELATION OF THE PROTEIN CONTENT OF HARD RED SPRING WHEAT WITH PHYSICAL CHARACTERISTICS AND BAKING QUALITY. Cereal Chem. 2: 107–112. 1925.

FREQUENCY DISTRIBUTIONS OF SAMPLES ON THE BASIS OF DARK-KERNEL CONTENT

A study of frequency distribution of samples on the basis of dark-kernel content has brought out some interesting facts.

TABLE 2.—*Frequency distribution of samples of hard red spring wheat on the basis of dark-kernel content*

Class[a]	1922 crop		1923 crop		1924 crop		1925 crop	
	Fre-quency	Percent-age of total samples	Fre-quency	Percent-age of total samples	Fre-quency	Percent-age of total samples	Fre-quency	Percent-age of total samples
10	0		2	1.00	12	3.79	0	
20	0		5	2 51	8	2.53	0	
30	0		7	3 51	6	1.89	2	0 45
40	2	2 22	13	6 53	6	1.89	5	1. 14
50	3	3 33	14	7.03	12	3.79	12	2. 75
60	8	8.88	20	10 05	13	4. 11	21	4.81
70	10	11 11	25	12 56	31	9.81	21	4. 81
80	16	17.77	38	19 09	53	16 77	47	10 77
90	29	32 22	50	25. 12	109	34.49	77	17. 66
100	22	24.44	25	12 56	66	20.88	251	57. 56
Total	90		199		316		436	

a The class mark denotes the mid-point of the group. For example, the 80 class includes all samples containing from 75 to 85 per cent kernels, the last class (100) includes samples containing from 95 to 100 per cent of dark kernels.

Table 2 gives frequency distribution on the basis of dark-kernel content for samples from the four crops studies. Figures 1 and 2 show distribution for the crops of 1924 and 1925. It will be noted from Table 2 that in all the crop years recorded more than half the samples were found in the three highest classes; which means that when graded more than half of the total samples would fall into the dark northern spring subclass. The data in Table 2 were obtained through crop surveys and, in the writer's opinion, represent a fairly accurate cross section of the North Dakota crop for the years given. Table 2 indicates that in 1925 over 85 per cent of the North Dakota crop would fall in the dark northern spring subclass, that is, it contained 75 per cent or more of dark kernels.

The frequency distributions of samples on the basis of dark-kernel content when plotted give, with one exception, the same type of curve.[5] The frequency distribution curves from the crops of 1922, 1923, and 1924 can be shown by calculation to be Pearson's normal type I curve, and in all cases are skewed to the right. The data for 1925 give a Pearson's J-shaped type I curve. All the distribution curves show similarity in that they are skewed to the right.

FREQUENCY DISTRIBUTIONS OF SAMPLES ON THE BASIS OF PROTEIN CONTENT

The frequency distributions of samples on the basis of protein content are shown in Table 3 and in Figure 3. For three of the four years (1922, 1924, and 1925) the greatest frequency is found at 11 per cent. The frequency distribution curves for these data (fig. 3) can be shown by calculation to be Pearson's type I curve, that is,

5 PEARSON, K. TABLES FOR STATISTICIANS AND BIOMETRICIANS. PART I. Ed. 2, 143 p., illus. [Cambridge, Eng. 1924.]

with the exception of the 1924 crop, in which the distribution apparently follows Pearson's type VI curve. The curves for these distributions show similarity in that they are in all cases skewed to the
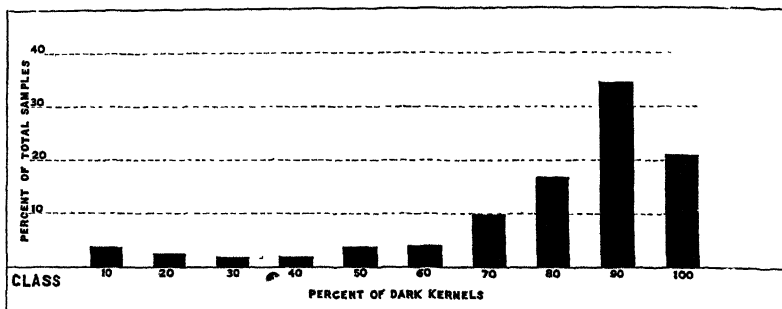
FIG. 1.—Distribution on the basis of dark-kernel content of samples from North Dakota spring-wheat crop for 1924

left. The 1923 data show the least and the 1925 the greatest skewness. The frequency distributions for protein content show appreciable skewing to the left, but as has been previously pointed out, the frequency distributions on the basis of dark-kernel content
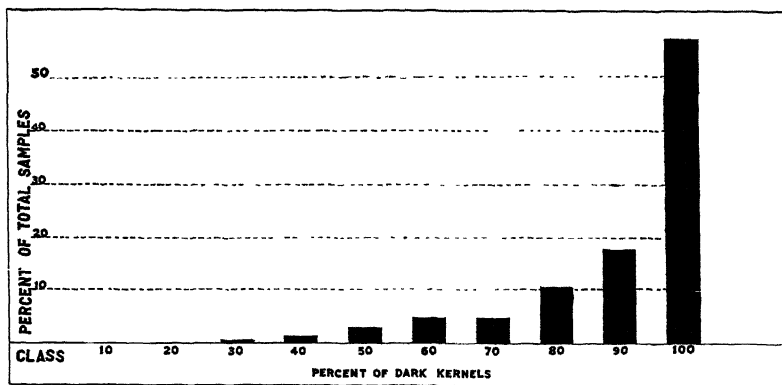
FIG. 2.—Distribution on the basis of dark-kernel content of samples from North Dakota spring-wheat crops, 1925

in all cases were skewed to the right. If a high degree of correlation existed between protein content and dark-kernel content, one would expect to find the distributions skewed in the same direction.
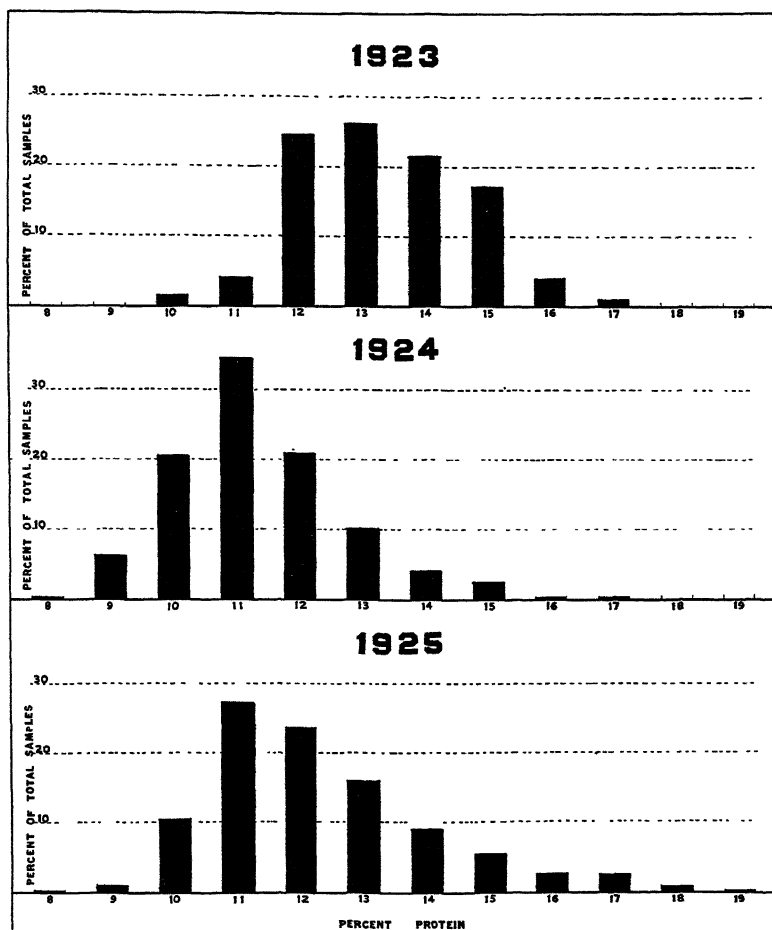
FIG. 3.—Distribution on the basis of protein content of samples from North Dakota spring-wheat crops, 1923-1925

TABLE 3.—*Frequency distribution of samples of hard red spring wheat on the basis of protein content*

Class a	1922 crop		1923 crop		1924 crop		1925 crop	
	Frequency	Percentage of total samples	Frequency	Percentage of total samples	Frequency	Percentage of total samples	Frequency	Percentage of total samples
8	0	----------	0	----------	1	0 32	1	0. 23
9	3	3. 33	0	----------	20	6. 33	4	. 92
10	11	12. 22	3	1. 51	65	20. 57	45	10. 32
11	24	26. 67	8	4. 02	109	34. 49	119	27. 41
12	16	17. 78	49	24. 62	66	20. 89	104	23. 74
13	17	18. 87	52	26. 13	32	10. 12	70	16 06
14	14	15. 56	43	21. 61	13	4. 11	40	9. 17
15	2	2. 22	34	17. 09	8	2. 53	24	5. 50
16	2	2. 22	8	4. 02	1	. 32	12	2 75
17	1	1. 11	2	1. 01	1	. 32	12	2. 75
18	0	----------	0	----------	0	----------	4	. 92
19	0	----------	0	----------	0	----------	1	. 23
Totals	90	----------	199	----------	316	----------	436	----------

a The class mark denotes the mid-point of the class. For example, the 12 per cent class includes all samples which fall within the range of 11.5 to 12 5 perent protein.

PROTEIN VARIATION AND DARK-KERNEL CONTENT

The wide variation in protein content of samples showing a high percentage of dark kernels was discussed briefly under correlation studies. Figures 4, 5, and 6 show the spread in protein content for (1) all samples, (2) samples containing more than 75 per cent of dark kernels, and (3) samples containing more than 85 per cent of dark kernels.
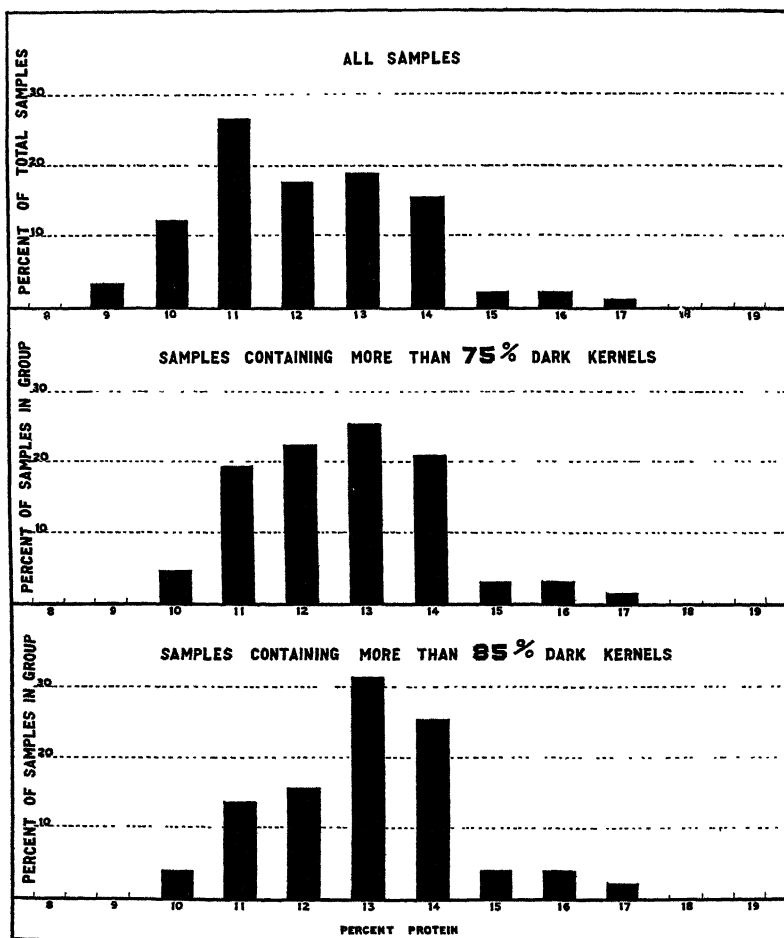
Figures 4, 5, and 6 show that the spread in protein content in groups containing more than 75 per cent or more than 85 per cent of dark kernels is almost as large as the protein spread for all samples. In 1925 the protein content for all samples varied from 8 to 19 per cent, as shown by Figure 3, but the spread for the 75 and 85 per cent dark-kernel groups was almost as great—9 to 19 per cent. In 1924 the protein varied in all samples from 8 to 17 per cent, and the variation

for the 75 and 85 per cent dark-kernel groups was from 9 to 17 per cent. The 1923 crop averaged higher in protein than the 1924 and 1925 crops, but the spread in protein content for all samples was from 10 to 17 per cent, and the variation in the 75 and 85 per cent dark-kernel groups was from 11 to 17 per cent. The 1922 crop shows a greater degree of correlation between dark kernels and protein content
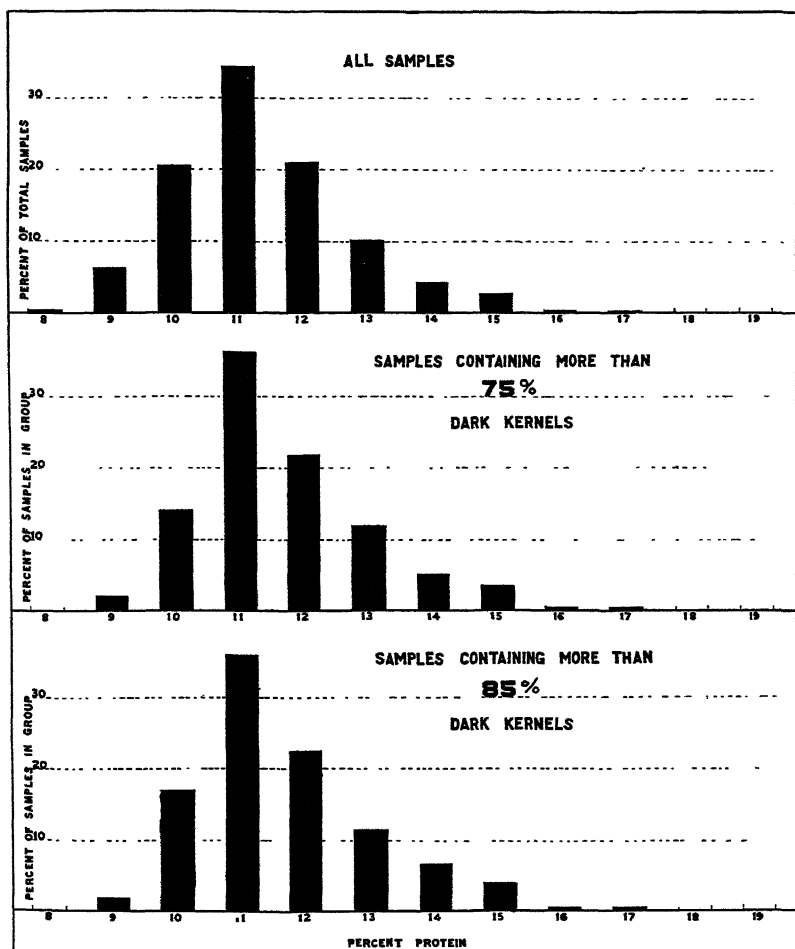
Fig. 5.—Distribution on the basis of protein content of samples from North Dakota spring-wheat crop for 1924

than other crops studied, but the spread in protein content for all samples of the 1922 crop was from 9 to 17 per cent, and the variation in the 75 and 85 per cent dark-kernel groups was from 10 to 17 per cent.

For the 1924 and 1925 crops the greatest frequency (figs. 5 and 6) for all samples is found in the 11 per cent class, but the greatest frequency for the 75 and 85 per cent groups is also found in the 11 per cent class.

For the 1923 crop the greatest frequency is found in the 13 per cent class for all samples, but the greatest frequency for the 75 per cent dark-kernel group again occurs in the same class (13 per cent).

The 1922 crop (fig. 4), as would be expected from the correlation coefficient, differs quite distinctly from the other crops. The greatest frequency for all samples of the 1922 crop occurs in the 11 per cent
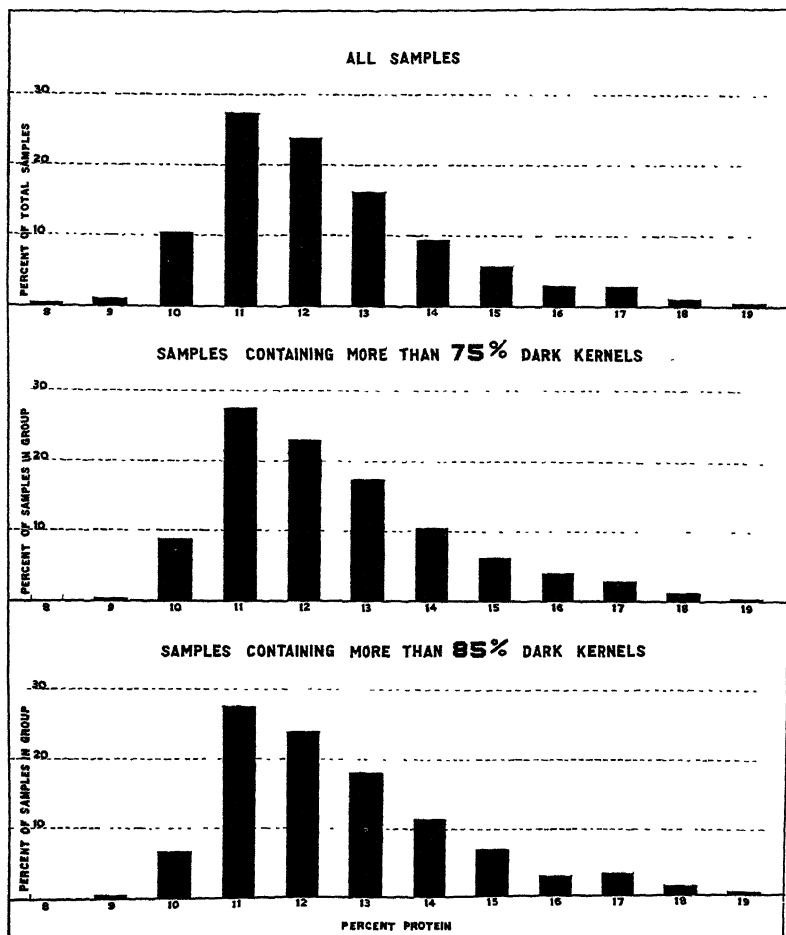
FIG. 6.—Distribution on the basis of protein content of samples from North Dakota spring-wheat crop for 1925

class, and in this respect the crop is similar to the 1924 and 1925 crops, but the greatest frequency for the 75 per cent and 85 per cent dark-kernel groups is found in the 13 per cent class.

In Table 4 the samples are grouped in such a way as to bring out the relation of dark-kernel protein content to protein content of all samples. As will be noted, the 1922 crop shows a larger increase in protein for the 75 and 85 per cent dark-kernel groups than any crop of the other three years.

TABLE 4.—*Comparison of mean protein content of dark kernels and of kernels of all samples in hard red spring wheat, 1922–1925*

| Year | Mean protein content of— | | |
	All samples	Samples containing more than 75 per cent of dark kernels	Samples containing more than 85 per cent of dark kernels
	Per cent	*Per cent*	*Per cent*
1922	12. 12	12. 67	12. 98
1923	13. 35	13. 48	13. 45
1924	11 35	11. 56	11 66
1925	12. 31	12. 43	12. 53

In the 1922 crop there is apparent a significant degree of correlation between dark kernels and protein content, but it was in 1922 that the protein test was first used extensively on the Minneapolis market. The 1922 crop shows a higher degree of correlation than the crops which followed, but even with this crop, the dark-kernel content was not considered sufficiently accurate as an index of protein content, and the grain trade began to use the protein test. The dark-kernel content is apparently of very questionable value in estimating the potential baking value of a lot of wheat. A high percentage of dark kernels does not assure the wheat buyer that he is getting a wheat that is high in protein, and for this reason the protein test is now considered by many buyers of equal importance with the grade certificate.

SUMMARY

Correlation between dark kernels and protein content shows considerable seasonal variation, and the degree of correlation may be quite low.

Frequency distributions of representative samples from North Dakota spring-wheat crops indicate that in every year of the four recorded a large proportion of the crop contained a high percentage of dark kernels and would fall in the highest subclasses when graded.

Frequency distributions of samples on the basis of dark-kernel content are skewed to the right, while distributions on the basis of protein content are skewed to the left, a fact which indicates a lack of close relationship between dark kernels and protein content.

Frequency distributions of samples containing more than 75 per cent or more than 85 per cent of dark kernels, respectively, when compared to distributions for all samples show (1) that total spread or variation in protein content is almost as great in 75 per cent or 85 per cent dark-kernel groups as in the total samples; (2) when distributions of all samples are compared with distributions of samples containing more than 75 per cent or more than 85 per cent of dark kernels, the greatest frequency falls in the same class in all groups, except in the case of the 1922 crop, where the greatest frequency moves up two classes—that is, into the 75 and 85 per cent

dark-kernel groups; (3) the mean of samples containing 85 per cent and 75 per cent, respectively, of dark kernels is very little larger than the mean of all samples except in 1922, when a significant increase is noted.

The color of the kernel is not a sufficiently accurate index of protein content to be used by the wheat buyer, and for this reason is being superseded by actual protein test. As an index of wheat quality, dark-kernel content is of questionable value.

THE COMPUTED AS COMPARED WITH THE DIRECTLY OBSERVED FASTING KATABOLISM OF CATTLE AS A MEASURE OF THE MAINTENANCE REQUIREMENT OF ENERGY [1]

By E. B. FORBES, *Director*, MAX KRISS, *Associate*, and WINFRED W. BRAMAN, *Associate, Institute of Animal Nutrition, Pennsylvania State College*

INTRODUCTION

The maintenance requirement of energy is the amount that is necessary to keep an animal in energy balance, that is, without energy gain or loss, under the standard conditions of activity to which the measure is to apply. This maintenance requirement is, therefore, a charge or expense which must be met before there can be a profitable product from feeding, and it is the most definite and practical fixed value in terms of which to reckon a rate of feed supply, that is, a plane of nutrition. It is also of special significance in the work of the Institute of Animal Nutrition of Pennsylvania State College, inasmuch as the main program of research, from the beginning, has been the determination of net energy values of feeds and net energy requirements of animals, in the ascertaining of both of which measurements separate estimations of the maintenance requirement of energy must be made. In view, further, of various special technical difficulties involved in determining the maintenance requirement, the principles and methods involved have been at all times matters of critical concern; and since there is occasion at this time to change the practice of the institute in determining the maintenance requirement of cattle it becomes desirable in this relation to review the development of the ideas held and methods followed by Armsby from the beginning of his respiration-calorimetric work at this institute in 1901 until his death in 1921.

DISCUSSION OF WORK OF ARMSBY AND OTHERS

The standard measure of the maintenance requirement of energy, with all species of animals, is the heat production during fast—a definition unequivocally accepted and at all times followed by Armsby, and discussed at length in United States Bureau of Animal Industry Bulletin 143 (*5, p. 41*).[2]

It is also a noteworthy fact that Armsby's standard for maintenance requirement of energy of swine, recommended by him in 1917 (*7*), is based entirely on measurements of the katabolism of fasting animals.

In the case of ruminants, the question has been not as to the standard but as to the method of measurement—as to whether the

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[1] Received for publication Oct. 27, 1926; issued February, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 178.

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fasting katabolism is to be determined directly, as the heat produc-
tion of a fasting animal, or, in view of the alleged impracticability of
the direct determination, whether it is to be computed from the
difference in heat production and difference in feed between two
periods differing as to feed intake.

Because of the great capacity and the complicated anatomy of the
ruminant alimentary tract and the slow and indirect course of the food
and its residues in digestion, Armsby assumed at the beginning of his
work in animal calorimetry that in the measurement of heat production
during fast the continued presence of feed residues in the alimentary
tract would affect the heat production, presumably either through
fermentation or through the utilization of nutrients, and therefore
that it is impracticable to determine directly the fasting katabolism
of cattle.

The plausibility of this assumption is indicated by the conclusions
of Ewing and Smith (*13*), who computed from the feed intake, the
rate of fecal outgo, and the contents of the alimentary tract of
steers, as determined at the slaughter of the animals after they had
been fed rations composed of different proportions of cottonseed meal
and corn silage, that the rate of passage of the residues through the
animals varied from 2.92 days to 5.24 days.

This fact, which is generally appreciated as a result of observations
of various kinds, is naturally discouraging to the idea of getting cattle
into a status of complete fast.

The writers will show, however, that these difficulties are readily
surmountable; that it is entirely practicable to get cattle into a
status of essentially complete fast; and that the direct measurement
of the heat production of cattle during fast affords a measure of the
maintenance requirement of energy which is more satisfactory on
several accounts, but especially because it is much less variable,
than is a computed value. In the development of this point of view
and with the same understanding of the problem, the writers are con-
cluding a study begun by Armsby, in the last experiment conducted
under his direction in 1920–21.

In the light of their earlier understanding of this situation, Armsby
and Fries wrote, in 1903 (*8*), in the report of their first year's calori-
metric experimentation on the available energy of timothy hay (ex-
periment 174), that it was impracticable to determine directly the
fasting katabolism—a view which Armsby expressed again and again
in later publications. The following statement by Armsby (*7, p. 285,
288, 290*) in 1917, expresses very clearly a late view which he held
on this matter:

With animals such as man, carnivora, or swine, having a comparatively simple
digestive apparatus and consuming relatively concentrated feed, the fasting
energy expenditure can be determined without special difficulty by depriving
the resting animal of feed during a relatively short period and measuring the
katabolism with the aid of a respiration apparatus or calorimeter. The total
amount of heat produced, determined either directly or by calculation, furnishes
the measure of the energy expenditure and therefore of the net energy requirement
for maintenance. * * *

In the case of ruminants it is hardly practicable to determine directly the net
energy requirement by measuring the katabolism of the fasting animal. Pro-
longed fasting would be required to free the voluminous and complicated digestive
organs of these animals from feed residues, if this could be accomplished at all,
and it would be difficult to determine when that point was reached, while it is
questionable whether the results on such an animal could be regarded as normal

The fasting katabolism of such an animal may, however, be computed in the manner already described (374) from a comparison of two periods on different amounts of the same feed, or ration, both being less than that necessary for maintenance.

In reiterating the last statement later (*12, p. 44*) and in the actual use of the indirect method of obtaining the fasting katabolism, Armsby's view with regard to this matter was modified by the omission of the previously prescribed condition that the rations compared be less than that required for maintenance. This omission signifies the acceptance of the view that the utilization of feed energy is the same above and below the maintenance level.

The writers recently discussed the possibility that the directly observed heat production during fast might not be "normal," (*18*) at such length as seems justified by the slight evidence available on the subject, and concluded that even if the heat production during fast should include a factor of heat production of "abnormal" origin, characteristic of fasting, this fact would be without effect on the significance of the heat production of fast in relation to the energy required for maintenance, since the heat production of fast is the *measure* of the maintenance requirement of energy and of the net energy value of the amount of feed necessary to keep the animal in energy equilibrium.

The following example is presented as an illustration of the simplest use of the computation method of determining the fasting katabolism or maintenance requirement.

Experimental periods	Live weight	Dry matter of feed eaten	Heat production	Heat increment per kg. dry matter	Mainte-nance re-quirement of net energy
	Kgm	*Kgm*	*Cals*	*Cals.*	*Cals.*
1	415 2	3, 6674	8, 224	706	5, 635
2	429. 7	5 4676	9, 495		
Difference		1 8002	1, 271		

The heat increment, or energy cost of feed utilization, is comprised of the aggregate expenditure of energy in prehension, mastication, deglutition, fermentation, rumination, peristalsis, digestion, transportation, dynamic stimulation, and excretion. It is computed by dividing the difference in heat production in the two periods by the difference in feed and multiplying the result by the dry matter of the feed. Then the maintenance requirement is computed by subtracting this total heat increment from the heat production. Thus, in this procedure, the heat production is computed to a basis of zero feed intake.

In the use of heat increments in determining the net energy values of feeds and the net energy requirements of animals a number of heat increments were usually computed from the several periods of an experimental program and used either individually or as an average (according to which of three methods was employed).

Two important weaknesses of the heat-increment method arise from the fact that each increment involves two experimental periods. Thus the resultant of the errors of heat measurement might be either

additive or compensating, and this distortion of values might be greatly magnified through their being referred to markedly different amounts of feed in the computation of the heat increments per unit of the ration.

These conditions have led to a most embarrassing diversity of heat-increment values, often derived from work which was really very good, and have required either a difficultly defensible averaging of discordant heat increments, or a more difficultly justifiable exclusion from the average of those heat increments which were most widely divergent from the average. In this situation it is obvious that the only other condition necessary to hopeless entanglement and confusion was the lapse of sufficient time to develop the effects of these inconsistencies.

A further difficulty in the heat-increment method of computation of the maintenance requirement seems to result from the assumption, which is fundamental to its use, that it varies directly as the dry matter of the feed. On account of the great variability of computed maintenance values and the fact that the computed maintenance from supermaintenance periods is always a materially lower value than is the directly determined fasting katabolism (compare Tables 1 and 2), the writers believe that the heat increment does not thus vary directly as the feed.

In order to appreciate the significance, consequences, and bearings of the facts as to the relation of the heat increment to the plane of nutrition, it is necessary to understand that the net useful energy of the ration, for any purpose, is the metabolizable energy (gross energy minus the energy of urine, feces, and methane) minus the heat increment.

The same principles, therefore, determine the utilization of the ration for maintenance as for production, and any use of heat increments determined at one level of feed intake in relation to the utilization of feed at another intake (as in the above numerical illustration) or the use of any single net energy value of a feed as applying to both maintenance and production, involves the assumption that the heat increment varies directly as the dry matter; that is, that it is a linear function of the amount of the feed.

In tracing the development and changes of Armsby's ideas as to the utilization of energy for maintenance, therefore, his observations on the utilization of energy for production will be considered as similarly relevant, since the heat increment is of like fundamental significance in both relations.

The following excerpts from and notes on the papers of Armsby and of Armsby and Fries are presented in chronological order. To avoid confusion, it is necessary to understand that Armsby used in his early writings the expression "net available energy," or sometimes simply "available energy," to mean net energy used for maintenance only, and spoke of the "utilization" of energy as meaning the use of energy over and above that required for maintenance for the production of body increase.

In 1903 Armsby and Fries (*8, p. 58, 64*) found the utilization ("availability") of metabolizable energy (feed minus excreta) to be 62.92 per cent on a plane of nutrition below maintenance, and 33.31 per cent for the production of body increase. They assumed that the net energy for maintenance and for production are *different linear*

--*functions* of the metabolizable energy. In the following paragraph this conclusion is given in the author's own words:

> Our results indicate that the proportion of the metabolizable energy of the food which was utilized, above the maintenance requirement, to produce gain was decidedly less than that used below the maintenance requirement to prevent loss of tissue. In other words, they indicate that the conversion of digested and assimilated matter into actual tissue (fat) requires a considerable expenditure of energy, amounting in this case to about 47 per cent of the available energy or 30 per cent of the metabolizable energy. This result is quite in accordance with what we should anticipate. The digested matter of the food of herbivora appears to be resorbed chiefly in the form of carbohydrates and of organic acids. It seems altogether probable that a much less profound change is required to convert these resorbed products into forms suited to maintain the energy metabolism of the organism than is needed to convert them into the form of permanent tissue, especially fat.

In another 1903 publication Armsby (*1, p. 430*) similarly discussed the influence of amount of food on availability.

In 1905 Armsby and Fries (*9, p. 7, 42–44, 47*) discussed the different rates of use of energy for maintenance and for production and called attention to the harmony between this conclusion and the generally accepted principles of the physiology of nutrition. These ideas were also restated in three other publications during the next two years (*4, p. 281, 282; 2, p. 11–12; 3, p. 15*).

In 1911 Armsby and Fries wrote (*10, p. 48–49*):

> In previous publications we have expressed the belief, based in part upon experimental results and in part upon theoretical considerations, that this is not the case [that the availability of energy is the same above and below the maintenance requirement][3] but that the proportion of metabolizable energy capable of being stored up as gain after the maintenance requirement is satisfied is materially less than that available below the maintenance requirement to diminish the loss of energy from the body. The results of the present series of expriments fail to support that view.

After citing conflicting evidence and discrepancies (which can not conveniently be quoted), in accounts of both this and the earlier experiments, Armsby and Fries said:

> We are inclined to conclude that the percentage of the metabolizable energy of the grain available was substantially the same above and below the maintenance requirement and that the discrepancies in the earlier experiments are perhaps due to errors.

This was the beginning of Armsby's abandonment of the idea that the heat increment represented different linear functions of the metabolizable energy above and below maintenance. In the same bulletin Armsby and Fries (*10, p. 59*) suggested that hay, as distinguished from grain, may be more efficiently utilized below than above the plane of maintenance.

Conclusions on this subject during the remainder of Armsby's life expressed the same uncertainty that characterized the paper written in 1911. This situation obviously resulted from numerous imperfections of methods, as revealed by recent studies (*14, 15, 16, 20, 21, 23*), but especially from an imperfect method of computing the heat production to the standard day as to standing and lying.

Further expressions of Armsby's changed conception as to the relation of the heat increment to the plane of nutrition are commented upon below.

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[3] Explanation in brackets is supplied by the authors of this paper.

In 1912 Armsby (*5, p. 9, 15, 36, 41, 42*) expressed continued adherence to the fasting katabolism as a measure of the maintenance requirement of energy, commented on its constancy as being in harmony with ideas expressed as to its origin and significance, and reaffirmed his belief that a larger percentage of the energy of hay is available below the point of maintenance than is utilized for gain above it.

In the bulletin on the maintenance ration of farm animals published in 1912, Armsby first described and used the heat-increment method of computing the maintenance requirement of net energy. In this relation he said:

The experiments just cited are the only ones thus far reported in which this precise method of determining the maintenance requirement in terms of available energy has been followed.

In 1915 Armsby and Fries (*11, p. 476*) said:

The data * * * fail to give any distinct evidence of a greater relative increase of heat production on heavy as compared with light feed or on supermaintenance as compared with submaintenance rations, the average tending, if anything, to be a trifle lower on the heavier rations.

Then, on account of the wide variability of the heat increments derived from the several periods of an experimental program, they expressed confidence in the increment derived from the greatest difference in feed, as minimizing the errors incident to the determination.

In 1916 Armsby (*6, p. 10*) reported net energy values primarily for maintenance or fattening, but said:

There seems good reason for believing, however, that they may be taken without serious error to represent also the net energy values for growth and at least the relative values for milk production. Kellner believes that the same net energy values may also be regarded as expressing with sufficient accuracy the relative values of feeding stuffs for horses and for swine.

This expression seems to mark the completion of the change, begun in 1911, from the idea of the heat increment as representing different linear functions of the metabolizable energy for maintenance and production to one of the heat increment as a single straight-line function of the same.

In 1917 Armsby (*7, p. 279, 361, 450*) expressed the idea that net energy values for maintenance and production "might very well differ."

Explaining the theoretical ground for his original conception of net energy values for fattening being lower than for maintenance, he continued:

Such data as are available, however, do not appear to indicate that this difference is a considerable one in the case of farm animals, and it would appear that, in the case of cattle at least and presumably in that of other species, the net energy values of feeding stuffs may be regarded as being substantially the same for fattening as for maintenance.

Further on, he said:

There appears to be a somewhat general impression, however, that in addition to this effect on digestibility, the matter and energy actually resorbed from the ration become less efficient in producing gain as the amount of the ration is increased—in other words, that when the organism is flooded with the resorbed products of digestion, the katabolic processes are stimulated and a larger share of the energy of the digested matter escapes as heat. As appears in Chapter

XVII (764), the evidence on this point as yet seems hardly sufficient to warrant positive statements. The net energy values of feeding stuffs which have thus far been reported have been obtained chiefly in experiments on rations ranging from submaintenance to only moderately heavy fattening rations, and the results show no distinct indication of a decrease with increasing amounts of feed. On the other hand, physiological considerations render it quite conceivable that the effect of the feed in stimulating metabolism and so increasing the heat production (365) may be relatively greater on a high than on a low nutritive plane.

In 1918 Armsby, Fries, and Braman (*12, p. 44*) said, referring to the heat-increment method of computing the maintenance requirement:

Strictly speaking, the foregoing method of computation assumes that the heat production caused by the feed is a linear function of its amount. This can not be regarded as having been proved, but no distinct indications to the contrary have appeared within the range of our experiments.

The article appearing in 1918 was Armsby's last published expression on this subject.

In Armsby's laboratory outline for experiment 221 E, the last research conducted under his direction, in 1920–21, occurred the following:

Incidentally the basal katabolism of the animals as computed by a comparison of two periods on different amounts of feed is to be compared with katabolism as measured directly by the heat production after a longer or shorter period of fasting in order to ascertain whether it is possible to determine the basal katabolism of cattle by a single determination of the heat production in what corresponds to the "post-resorptive" state in man.

In regard, then, to the fundamental principle involved in the subject of this paper, namely, the relationship of the energy cost of feed utilization—the heat increment—to the amount of the feed, Armsby expressed himself in the publications of this institute during the years 1903 to 1907, inclusive, as in favor of the idea that the heat increment varied directly as the metabolizable energy of the ration, but at different rates for maintenance and production.

Beginning with the year 1911, he tended toward the belief in a single heat-increment value for the whole range of variation in plane of nutrition, first for grain alone, and later for hay and grain together; he used an original method for computing the maintenance requirement in a way to involve the assumption of a single heat-increment value; but he repeatedly called attention to the theoretical ground for belief in higher utilization (lower heat increments) for maintenance than for production, though in his last paper he said, in effect, that the idea of a single heat-increment value applying to production and to maintenance had not been proved; and in the outline for his last calorimetric experiment he showed by his plan to compare the directly determined with the computed maintenance requirement (a difference between which would indicate different rates of utilization of energy at different planes of nutrition) that he regarded this heat-increment-maintenance problem as still lacking final solution.

The computation method of determining the maintenance requirement of energy was used in all of the reports of respiration calorimetric studies at this institute until December, 1925, when, as a result of recent studies, a change to the directly determined value was announced by Forbes.[3] Notice of this change was also published

[3] FORBES, E. B. ADVANCES IN RESPIRATION CALORIMETRY WITH CATTLE. Amer. Soc. Anim. Prod. Proc. 1925-26. (In press)

published in Science (*17*), and the data in three later papers (*17*, *18*, *19*) have been handled in the light of the new procedure.

The fact that the computed maintenance is significantly different from the directly determined value shows that energy is utilized for maintenance and for production at different rates of efficiency and, therefore, that the energy cost of feed utilization is not directly proportional to—that is, is not a linear function of—the dry matter or the metabolizable energy of the feed. The question as to whether the efficiency of utilization of energy for maintenance and for production can be represented as different linear functions of the dry matter or the metabolizable energy of the feed, as Armsby originally thought, or whether it is a curve, is not certain, and is being studied at this institute.

DATA SUPPORTING CHANGE IN METHOD

The following are the data on the strength of which the computation method has been abandoned in favor of the direct method of determining the fasting katabolism as a measure of the maintenance requirement of energy. The comparison of the computed and the directly measured katabolism discloses their respective degrees of variability and indicates the extent of their self-consistency and reliability.

Since the direct method has been adopted in principle only and has not been finally standardized, an arbitrary basis of comparison with the computation method was determined upon as the fairest practicable means of comparing the inherent variability of results obtained by the two methods.

Thus, in representing the direct method, the average heat production of each two consecutive days of fast was considered as an estimation of the maintenance requirement of energy, and for the indirect or computation method, the maintenance value derivable from each possible comparison of the heat production of two 2-day calorimeter periods was considered as a determination. On this basis 18 direct and 62 indirect determinations are compared.

In the actual use of heat increments in the computation method, as many determinations of the maintenance requirement are averaged as there are experimental periods; and in the use of the direct method the procedure will be standardized (as it has not yet been) in such a way that the heat production will be as nearly as possible that of a truly fasting condition.

The subjects in the last two experiments recorded in Table 1 (experiments 221D and 221F) were cows; in all the other experiments summarized in Table 1 the subjects were steers. In experiments 221D, 221E, and 221F of Table 2 the subjects were cows; in experiments 235 and 237 the subjects were steers.

TABLE 1.—*Computed fasting katabolism per 100 kilograms of live weight and per square meter of body surface*

Experiment No.	Animal	Periods compared	Average live weight	Computed fasting katabolism		
				Per head	Per 100 kgm live weight	Per square meter body surface
			Kgm	*Cals*	*Cals*	*Cals*
174	I (steer)	A and B	393	6,206	1,579	1,335
		B and C	408	7,806	1,913	1,643
		C and D	419	6,581	1,571	1,360
		C and A	400	6,771	1,693	1,441
		D and A	404	6,740	1,668	1,425
		D and B	412	7,360	1,786	1,540
179	I (steer)	1 and 2	533	6,617	1,241	1,177
186	I (steer)	2a and 3a	571	7,309	1,315	1,279
		2b and 3b	571	8,641	1,513	1,472
190	A (steer)	3 and 4	275	4,546	1,653	1,222
200	A (steer)	3 and 4	403	6,222	1,544	1,318
207	A (steer)	3 and 4	511	5,113	1,001	935
190	B (steer)	3 and 4	192	4,494	2,341	1,513
200	B (steer)	3 and 4	303	5,828	1,923	1,475
207	B (steer)	3 and 4	380	5,552	1,461	1,220
208	C (steer)	2 and 3	264	3,576	1,355	988
		4 and 5	286	3,706	1,296	973
		5 and 6	280	3,415	1,220	908
		6 and 4	282	3,540	1,255	937
208	D (steer)	1 and 2	167	2,549	1,526	937
208	E (steer)	1 and 2	208	3,756	1,806	1,204
		2 and 3	201	3,658	1,820	1,195
		3 and 1	203	3,704	1,825	1,207
		5 and 6	206	3,070	1,490	990
209	F (steer)	1 and 2	297	4,923	1,658	1,262
		2 and 3	288	4,872	1,692	1,275
		3 and 1	292	4,891	1,675	1,267
		5 and 6	300	5,027	1,676	1,282
211	D (steer)	2 and 3	451	6,067	1,345	1,199
		1 and 4	458	6,188	1,351	1,211
		4 and 5	442	6,678	1,511	1,336
		5 and 1	444	6,528	1,470	1,303
211	G (steer)	2 and 3	378	5,665	1,499	1,251
		1 and 4	388	6,108	1,574	1,325
		4 and 5	376	4,848	1,289	1,073
		5 and 1	377	5,269	1,398	1,163
212	H (steer)	1 and 3	352	4,628	1,315	1,066
		3 and 5	346	4,097	1,184	955
		5 and 2	343	4,210	1,227	986
		2 and 4	349	4,716	1,351	1,094
		4 and 6	339	2,807	828	662
		6 and 2	339	3,208	946	757
216	J (steer)	1 and 2	378	5,144	1,361	1,136
		2 and 3	377	6,393	1,696	1,411
		3 and 4	372	5,477	1,472	1,220
		3 and 1	388	3,855	994	836
		4 and 1	372	5,055	1,359	1,126
		4 and 2	361	4,953	1,372	1,126
		5 and 6	404	4,483	1,110	948
		6 and 7	390	4,796	1,230	1,038
		7 and 5	390	4,726	1,212	1,023
217	J (steer)	1 and 2	513	5,574	1,087	1,015
220	K (steer)	1 and 2	505	6,670	1,321	1,228
		3 and 4	502	6,390	1,273	1,181
		4 and 5	502	7,397	1,474	1,367
		5 and 3	490	8,253	1,684	1,548
235	259 (steer)	3 and 2	365	4,499	1,233	1,013
235	260 (steer)	3 and 2	384	4,636	1,207	1,012
221D	886 (cow)	1 and 2	412	3,936	955	823
		3 and 2	422	3,873	918	797
221F	874 (cow)	1 and 2	423	5,298	1,252	1,090
221F	887 (cow)	1 and 2	328	4,360	1,329	1,051

Coefficient of variation of computed fasting katabolism per square meter of body surface, 17.93± 1 12 per cent; per 100 kilograms live weight, 19.52± 1.23 per cent.

TABLE 2.—*Observed fasting katabolism per 100 kilograms of live weight and per square meter of body surface*

Experiment and animal No	Days of fast	Live weight	Heat production per standard day			Average heat production of 2 consecutive days	
			Per head	Per 100 kgm live weight	Per square meter body surface	Per 100 kgm live weight	Per square meter body surface
		Kgm.	*Cals.*	*Cals*	*Cals.*	*Cals.*	*Cals.*
Experiment 235							
Steer 260	Fourth	356	6,322	1,776	1,440	1,796	1,456
	5½ to 6½	354	6,430	1,816	1,471	1,827	1,479
	Seventh	351	6,453	1,838	1,487	1,805	1,459
	8½ to 9½	349	6,180	1,771	1,431		
Experiment 237							
Steer 254	Third	339	6,517	1,922	1,555	1,908	1,536
	Fourth	334	6,324	1,893	1,516	1,882	1,498
	Fifth	328	6,137	1,871	1,479	1,847	1,452
	Sixth	323	5,886	1,822	1,425		
	Eighth	359	6,614	1,842	1,463	1,802	1,429
	Ninth	357	6,290	1,761	1,395	1,731	1,368
	Tenth	354	6,022	1,702	1,341		
Steer 36	Third	341	6,466	1,896	1,558	1,884	1,496
	Fourth	334	5,920	1,772	1,433	1,778	1,426
	Fifth	327	5,831	1,783	1,419	1,771	1,396
	Sixth	320	5,626	1,758	1,372		
Steer 47	Fourth	347	6,664	1,920	1,564	1,896	1,537
	Fifth	342	6,403	1,872	1,510	1,889	1,514
	Sixth	336	6,403	1,906	1,517		
Experiment 221 D:							
Cow 886	Second	411	6,779	1,649	1,404	1,615	1,368
	Third	405	6,400	1,580	1,331		
Cow 885	Second	420	6,523	1,553	1,339	1,541	1,326
	Third	417	6,374	1,529	1,312		
Experiment 221 E							
Cow 885	Fifth	421	6,384	1,516	1,308	1,524	1,313
	Sixth	419	6,413	1,531	1,317		
Experiment 221 F.							
Cow 874	Eighth	395	6,906	1,748	1,469	1,723	1,446
	Ninth	393	6,669	1,697	1,422		
Cow 887	Eighth	301	5,778	1,920	1,455	1,967	1,483
	Ninth	297	5,982	2,014	1,511		

Coefficient of variation of directly observed fasting katabolism per square meter of body surface, 4.51±0.51 per cent, per 100 kilograms live weight, 6 65±0.76 per cent.

The experimental routine in the last two experiments mentioned was characterized by one noteworthy innovation—the introduction of the idea of special treatment of the experimental subject to bring about as promptly as possible a condition of true and complete fast.

For this purpose a physic was given to steer No. 260, after which roughage was withheld for one day and grain for one-half day; then the paunch was washed out by means of a stomach pump, and an enema was given. The withholding of roughage for one day seems to have permitted the paunch to clear itself, inasmuch as it appeared to be empty when the pump was used; hence in the preparation of steer No. 259 in experiment 235, and of the three steers (Nos. 254, 36, and 47) in experiment 237, the pumping out of the paunch was omitted. The three steers in experiment 237, therefore, were prepared exactly as was steer No. 259 in experiment 235.

This treatment was more than fairly satisfactory, as indicated by the decrease in feces passed and in methane produced, but inasmuch as further improvement seemed practicable, an effort is being made in experiments now in progress to improve this preliminary treatment by increasing the length of time during which roughage is withheld— the animal being fed grain alone in the meantime.

A further detail of the procedure adopted in the standardization of the treatment preliminary to the determination of the energy metabolism of fast is invariably to have a period of feeding on a maintenance or energy-equilibrium basis immediately preceding the period of fast, thus regularizing the conditions with reference to a possible carrying over into the period of fast of an influence of the preceding plane of nutrition. This procedure was followed in all experiments with both cows and steers recorded in Table 2—that is, the plane of nutrition preceding the fasting period was one of approximate energy balance.

Table 1 shows that the coefficient of variability among 62 computed maintenance requirements was 17.93 ± 1.12 per cent, in terms of square meters of body surface, computed according to Moulton (*22*) and 19.52 ± 1.23 per cent, in terms of kilograms of live weight. These values varied from 662 to 1,643 Calories per square meter of body surface, the largest value being 248 per cent of the smallest.

Obviously such gross inherent variability renders the computation or heat-increment method of determination of the maintenance requirement highly unsatisfactory, and no method of treatment of the data which suppresses such extensive variability can be justified.

Table 2 shows that the coefficient of variability among 18 direct determinations of the maintenance requirement was 4.51 ± 0.51 per cent, in terms of square meters of computed body surface, and 6.65 ± 0.76 per cent, in terms of kilograms of live weight. These values varied from 1,313 to 1,537 Calories per square meter of body surface, the largest value being 117 per cent of the smallest.

The extreme variation of the computed maintenance requirement of an individual animal during the same season is from 836 to 1,411 Calories per square meter of body surface (experiment 216), the largest value being 169 per cent of the smallest, while in the direct determinations such variation is from 1,368 to 1,536 Calories (experiment 237, steer 254) the largest value being 112 per cent of the smallest.

These data show that the directly determined maintenance requirement (fasting heat production) is a fairly satisfactory measure, and further experiments which are in progress are expected to make possible such a standardization of the method of making this estimation as will still further reduce its variability.

CONCLUSIONS

A comparison is made of (1) 62 determinations of the maintenance requirement of energy for cattle, obtained by the heat-increment method, in which the heat production at a status of zero feed intake is computed from the observed heat production and feed intake at two planes of nutrition, and (2) 18 determinations, as the directly observed heat production during fast.

The computed determinations varied between 662 and 1,643 Calories per square meter of body surface, the coefficient of variation being 17.93 ± 1.12 per cent in terms of square meters of body surface and 19.52 ± 1.23 per cent in terms of the live weight.

The direct determinations varied between 1.313 and 1.537 Calories per square meter of body surface, the coefficient of variation being 4.51 ± 0.51 per cent in terms of square meters of body surface and 6.65 ± 0.76 per cent in terms of the live weight.

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The fact that the maintenance requirements as computed from supermaintenance rations are always lower than the directly determined values indicates that the relation between the heat production and the feed intake would not be represented graphically by a straight line and that the utilization of feed energy for maintenance is at a higher rate of efficiency than is its utilization for body increase.

On account of the narrower range of variation of the direct determination it has been adopted as the standard measure of the maintainance requirement of energy in place of the heat-increment method formerly used, and the standardization of this method is in progress.

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THE INHERITANCE OF LENGTH OF STYLE IN BUCKWHEAT[1]

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INTRODUCTION

The flowers of buckwheat (*Fagopyrum esculentum*) are dimorphic. One plant may have flowers with long styles—that is, the styles extend above the stamens, and another may have flowers with short styles. This relationship facilitates cross-pollination by insects, particularly honeybees, which visit the buckwheat flowers in great numbers. The investigation reported here had for its object the determination of the inheritance of length of style.

MATERIAL AND METHODS

Self-fertilization of Japanese and Silverhull buckwheat was induced by covering flower clusters with glassine bags or by covering entire plants with muslin cages. The selfed seed of each individual plant was kept separate and planted in a short row the next year. The seed was spaced along the row so as to permit individual plant study. The length of style of the parent plant and that of the progeny were noted.

In addition to the selfed seed a few seed produced by controlled crossing were obtained. The flowers with similar style lengths on different plants of the same variety were used in making the crosses. A few flowers only were cross-pollinated, but no particular difficulty was experienced in obtaining seed.

EXPERIMENTAL RESULTS

In Table 1 are listed the variety, plant number, year grown, style length of the parent, and number of plants in the progeny with long styles and with short styles, for the material studied during the four years of the experiment. In all, there were examined for style length the progeny of 29 plants that produced one or more selfed seed. The progenies of three plants on which were produced a few seed by artificial crossing were also examined for style length.

Of the self-fertilized plants of Silverhull 10 had short styles, each of which produced progeny that segregated with respect to style length. In all, the progeny consisted of 86 short-styled and 32 long-styled plants. The theoretical ratio on the basis of monohybrid segregation is 88.5 short to 29.5 long. The agreement between the actual and theoretical ratio is close. (Dev./P. E. = 0.8.) Of the

selfed plants of the Japanese variety 4 had short styles that segregated and produced a total of 19 short-styled and 8 long-styled plants. Here again the agreement between observed and theoretical ratio is close. (Dev./P. E. = 0.8.)

TABLE 1.—*The inheritance of length of style in buckwheat, Fagopyrum esculentum*

Variety	Plant No.	Year grown	Style length of parents	Number of plants in progeny with—	
				Long styles	Short styles
Japanese	1	1923	Long	8	0
Do	2	1923	do	2	1
Do	3	1923	do	13	0
Do	4	1923	Shoit	1	3
Do	5	1923	do	1	3
Silverhull	6	1923	do	1	0
Do	7	1923	do	1	7
Do	8	1923	do	2	0
Do	9	1923	do	1	7
Do	10	1923	do	4	2
Do	11	1923	do	1	3
Japanese (Long > Long)	12	1923	Long	2	0
Japanese (Short × Short)	13	1923	Short	3	4
Silverhull (Short × Short)	14	1923	do	1	5
Silverhull	15	1924	do	3	10
Do	16	1924	do	8	36
Japanese	17	1925	Long	1	0
Do	18	1925	do	11	1
Do	19	1925	do	0	1
Do	20	1925	do	0	1
Do	21	1925	Short	2	6
Do	22	1925	do	4	7
Silverhull	23	1925	Long	4	0
Do	24	1925	do	7	0
Do	25	1925	do	8	4
Do	26	1925	Short	1	0
Do	27	1925	do	3	4
Do	28	1925	do	7	5
Do	29	1925	do	0	1
Do	30	1925	do	4	8
Do	31	1925	do	0	2
Do	32	1925	do	3	4

Two of the Silverhull short-styled plants (Nos. 29 and 31) produced only short-styled progeny. This fact is of little significance, since the progeny consisted of only three individuals. None of the short-styled plants of Japanese produced exclusively short-styled progeny.

On the assumption that long style is recessive, one would expect the progeny of plants with that character to breed true. In general this expectation was fulfilled, although several exceptions occurred. Perhaps the most outstanding exception was plant 25 of the Silverhull variety, which produced eight long-styled and four short-styled plants. In each of several other cases there was one short-styled individual among the progeny of a long-styled plant The short-styled plants may have resulted from accidental cross-pollination, although considerable precaution was taken to prevent it. It is also possible that genetically long-styled plants may, under certain environmental conditions, become short styled.

The crossed seed produced on plants 12, 13, and 14 gave results in accordance with the assumption that short style is dominant to long style and that a single factor difference controls the inheritance of this character.

The results reported above are in agreement with those obtained by Althausen (1908),[2] Dahlgren (1922),[3] and Egiz (1925).[4]

CONCLUSIONS

The inheritance of style length in *Fagopyrum esculentum* is apparently controlled by a single-factor difference, short styles being dominant to long styles.

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SELF-FERTILIZATION IN BUCKWHEAT[1]

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INTRODUCTION

The improvement of buckwheat by breeding has not been attempted as extensively as has the improvement of other commonly cultivated field crops, probably because buckwheat is of minor economic importance in the world's agriculture. It is of considerable importance, however, in certain regions owing to the fact that it will yield greater returns on stony, unproductive soil and under cool climatic conditions than will most staple crops.

Two species of buckwheat are cultivated—*Fagopyrum esculentum* and *F. tartaricum*. Most of the buckwheat grown in the United States belongs to the former species. Some investigators name a third species, *F. emarginatum*, but others prefer to consider this form a variety of *F. esculentum*. From the genetic standpoint there is some evidence in support of the latter view.

The appearance of the flowers in the different species of buckwheat is decidedly dissimilar. *Fagopyrum esculentum* and *F. emarginatum* have somewhat showy flowers, which are distinctly dimorphic, whereas *F. tartaricum* has rather insignificant flowers, which are not dimorphic. The flowers of the two species first named are visited much more frequently by insects, particularly honeybees, than are the flowers of *F. tartaricum*. With regard to the number of chromosomes the three species are apparently alike. One of the authors (6)[2] has found the diploid number to be 16.

In 1921 an experiment in buckwheat breeding was begun at the West Virginia Agricultural Experiment Station. It was thought necessary first to determine the relative self-fertility of different kinds of buckwheat and to work out a satisfactory method of inducing self-fertilization by artificial means. The purpose of the present paper is to report the results of this phase of the investigation.

MATERIAL AND METHODS

Seed of different varieties of buckwheat was obtained from the United States Department of Agriculture, the University of Minnesota, and farmers in West Virginia. Each lot of seed was planted in a separate row and in a manner such that individual plants could be easily distinguished. The plants which were selfed by bagging flower clusters were spaced at intervals of about 2 inches in rows 1 foot apart, and those which were selfed by covering the entire plant with muslin cages (fig. 1) were spaced at intervals of approximately 30 inches in rows 2 feet apart.

Three methods of inducing self-fertilization were tried: (1) Flower clusters were covered with glassine bags just large enough so that each bag covered snugly but one flower cluster; (2) flower clusters were covered with glassine bags large enough to include several flower clusters from the same plant under one bag; (3) entire plants were covered with muslin cages. Some of the covered flowers were manipulated by rubbing between the thumb and forefinger without removing the glassine bags, and others were actually self-pollinated by rubbing the ripened anthers of a certain flower on the stigmatic surface of the same flower.

The glassine bags used for covering the flower clusters were made of high-grade paper, the edges of which were fastened with waterproof glue. The cages were constructed by building frames of suitable

FIG. 1.—Muslin cages used to cover individual buckwheat plants

dimensions and covering them with good-quality muslin. Some idea of their construction may be gained by examining Figure 1. After each muslin cage was placed in position over a single plant, a small ridge of soil was thrown up around the lower edge of the cage to exclude insects.

RELATIVE SELF-FERTILITY OF FAGOPYRUM ESCULENTUM AND F. TARTARICUM

The work of Althausen (*1, 2, 3*), Stevens (*7*), Dahlgren (*4*), Egiz (*5*), and others has proved beyond doubt that it is possible to self-fertilize *Fagopyrum esculentum*. The investigator first named pointed out that *Fagopyrum tartaricum* was spontaneously self-fertile. Similar results have been obtained at the West Virginia Agricultural Experiment Station.

In Table 1 are shown the different varieties, the year grown, the number of plants selfed, the number of plants which set seed, the total number of seeds set, and the range in number of seeds set per plant. The plants were selfed in various ways, but most of them were selfed by covering one or more flower clusters with a large glassine bag. The results, however, are comparable, as all the strains were treated approximately the same, except that in only a few instances were cages placed over plants belonging to the *tartaricum* species.

It is at once apparent from Table 1 that varieties of *tartaricum* set seed in abundance. In fact no appreciable difference in quantity of seed set was noted between flower clusters which were covered and those which were not. Sixteen plants of Mountain (Cereal Investigation No. 91) set a total of 817 selfed seed in 1921, and in most cases only one or two flower clusters were covered on a plant. The next year 8 of 10 plants of this variety set a total of 256 selfed seed. Two strains of rye buckwheat and a sort grown under the name of Tartarian also set considerable selfed seed in 1922. These varieties all belong to *Fagopyrum tartaricum*.

TABLE 1.—*Number of selfed seeds set by different varieties of two species of buckwheat*

Variety	Species	Year grown	Number of plants selfed	Number of plants setting seed	Total number of seeds set	Number of seeds set per plant
Mountain (C. I. 91)	Fagopyrum tartaricum	1921	16	16	817	10 to 119
Japanese	Fagopyrum esculentum	1921	27	2	6	1 to 5
Silverhull	do	1921	14	1	1	1
Mountain (C. I. 91)	Fagopyrum tartaricum	1922	10	8	256	
Rye (C. I. 79)	do	1922	6	5	211+	
Rye (Minnesota)	do	1922	8	7	197	
Tartarian (Minnesota)	do	1922	11	10	108	
Japanese	Fagopyrum esculentum	1922	52	11	61	1 to 21
Silverhull	do	1922	60	10	45	1 to 20
Gray (Minnesota)	do	1922	16	3	40	1 to 32

The results of self-fertilizing Japanese, Silverhull, and Gray buckwheat, all varieties of *Fagopyrum esculentum*, are also shown in Table 1. In 1921, of 27 selfed Japanese plants, 2 set a total of 6 seeds, and of 14 selfed Silverhull plants only 1 set 1 seed. In 1922 there were selfed 52 Japanese, 60 Silverhull, and 16 Gray plants, of which 11 Japanese, 10 Silverhull, and 3 Gray produced a total of 61, 45, and 40 seeds, respectively. A few of these selfed plants produced more than 20 seeds each, as may be seen from the last column of Table 1. It is obvious from the above-mentioned facts that although varieties of *F. esculentum* do not set selfed seed as abundantly as varieties of *F. tartaricum*, nevertheless, they will set some seed under conditions which induce self-fertilization.

METHODS OF SELFING

In 1922 the relative efficiency of large and small glassine bags over flower clusters and of muslin cages over entire plants in bringing about self-fertilization was determined. The data are presented in Table 2.

TABLE 2.—*Number of seeds of buckwheat (Fagopyrum esculentum) produced by different methods of selfing in 1922*

Variety	Flower clusters under—						Plants under muslin		
	Large glassine bags			Small glassine bags					
	Number of plants	Number of plants setting seed	Total number of seeds	Number of plants	Number of plants setting seed	Total number of seeds	Number of plants	Number of plants setting seed	Total number of seeds set
Japanese	27	6	16	16	0	0	9	5	45
Silverhull	31	3	15	20	0	0	9	7	38
Gray	10	1	1	4	0	0	2	2	39

Flower clusters on 27 Japanese, 31 Silverhull, and 10 Gray plants were covered with large glassine bags. Of these, 6 Japanese, 3 Silverhull, and 1 Gray set a total of 16 seeds, 15 seeds, and 1 seed, respectively. Under similar conditions flower clusters on 16 Japanese, 20 Silverhull, and 4 Gray plants were covered with small glassine bags, but not a single seed was obtained. The results with muslin cages were more satisfactory. Fourteen of the 20 plants covered set a total of 122 seeds. When one considers the fact that the muslin cages covered entire plants and the large glassine bags covered only a few flower clusters on each plant, the difference, if any, in the efficiency of the two methods in producing self-fertilization is not very striking. Moreover, the use of muslin cages is rather expensive and only relatively few plants may be covered.

Some of the flowers which had previously been covered were manipulated by being rubbed gently between the thumb and forefinger. No striking increase in the number of seeds set resulted from this treatment. Other flowers were selfed by rubbing ripened anthers on the stigmas by means of a tweezer or small swab. The swab was made by wrapping a bit of cotton around the end of a toothpick. Some seeds were set as a result of this treatment but not enough to justify its use for producing selfed seed in quantity.

RELATIVE SELF-FERTILITY OF LONG-STYLED AND SHORT-STYLED PLANTS

In order to determine whether length of style was correlated with the number of selfed seeds set in varieties of *Fagopyrum esculentum* the data presented in Table 3 were collected during 1922, 1923, 1924, and 1925. The variety, the year grown, the total number of plants selfed, the number that set seed, the total number of seeds, and the range in number of seeds set per plant by long-styled and short-styled plants are shown.

During the four years included in the experiment three varieties were under observation. Of 124 long-styled plants which were selfed, 22 set a total of 107 seeds, and of 116 short-styled plants, 40 set a total of 281 seeds. If only the plants that set seeds are counted, the short styled plants average approximately 7 seeds per plant and the long-styled plants 5 seeds per plant. Moreover, the relative number of plants that set selfed seed among the short-styled plants

was approximately double that among the long-styled plants. In this experiment it was apparently somewhat easier to obtain selfed seed from short-styled plants than from long-styled plants. In flowers of the former the stigmatic surface is beneath the anthers, whereas in flowers of the latter the positions are reversed. The chances that pollen will reach the stigmas of covered flowers on short-styled plants are somewhat greater than that it will reach covered flowers of long-styled plants.

TABLE 3.—*Number of selfed seeds produced by buckwheat plants (Fagopyrum esculentum) with long and with short styles*

Variety	Year grown	Long styles				Short styles				Method of selfing
		Total number of plants selfed	Number of plants setting seed	Total number of seeds	Number of seeds set per plant	Total number of plants selfed	Number of plants setting seed	Total number of seeds	Number of seeds set per plant	
Japanese	1922	16	3	28	2 to 21	20	7	25	1 to 12	Some plants covered entirely with a muslin cage; on others only a flower cluster covered with a glassine bag.
Silverhull	1922	28	5	8	1 to 3	20	5	37	1 to 20	
Gray	1922	11	1	7	7	3	2	33	1 to 32	
Japanese	1923	4	1	1	1	5	3	5	1 to 2	Each plant covered with a muslin cage.
Silverhull	1923	5	0	0	0	6	2	64	13 to 51	
Gray	1923	------	------	------	------	1	1	3	3	
Japanese	1924	13	2	2	1	13	1	2	2	Flower clusters only covered with glassine bags.
Silverhull	1924	16	1	1	1	21	6	60	1 to 29	
Japanese	1925	12	4	20	1 to 16	10	4	16	1 to 10	Do.
Silverhull	1925	19	5	40	2 to 19	17	9	36	1 to 12	Do.
Total	--------	124	22	107	--------	116	40	281	--------	

RELATIVE SELF-FERTILITY OF JAPANESE, SILVERHULL, AND GRAY BUCKWHEAT

Table 3 shows the relative self-fertility of the different varieties of *Fagopyrum esculentum*. In all there were selfed 93 plants of Japanese, 132 of Silverhull, and 15 of Gray buckwheat. Of these, 25 Japanese, 33 Silverhull, and 4 Gray plants set a total of 99, 246, and 43 seeds, respectively. The 4 plants of Gray which set seed averaged about 11 seeds per plant. The number of plants worked with in this variety was not sufficient for a random sample. Of the plants which set selfed seed in the other two varieties, Japanese averaged about 4 seeds per plant and Silverhull about 7 seeds per plant. These data indicate that the flowers of Silverhull when covered set somewhat more seed than do the flowers of Japanese when covered.

SUMMARY AND CONCLUSIONS

Several varieties of *Fagopyrum esculentum* and *F. tartaricum* were self-fertilized. Varieties of the latter proved much more highly self-fertile than varieties of the former.

Different methods were used in covering the flowers and in inducing self-fertilization. Large glassine bags and muslin cages were

found to be most satisfactory. The manipulation in various ways of previously covered flower clusters did not result in a marked increase in the amount of selfed seeds set.

The relative self-fertility of short-styled and long-styled plants of *Fagopyrum esculentum* as measured by the number of selfed seeds set was determined. Short-styled plants proved to be somewhat more self-fertile than long-styled plants.

In the experiment described in this paper relatively more selfed seeds were produced by Silverhull buckwheat than by Japanese.

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EFFECT OF SMUT (USTILAGO ZEAE) ON THE SUGAR CONTENT OF CORNSTALKS[1]

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INTRODUCTION

It has recently been shown (*3*) [2] that the specific gravity of the juice from cornstalks infected with *Ustilago zeae* is lower than that of the juice of comparable healthy stalks. The further observation that the juice from sound stalks is sweeter than that from diseased stalks suggested that the lower specific gravity of the juice from the diseased stalks is brought about by the removal of sugar by the smut fungus. This suggestion seemed all the more probable since it appears from the analyses given by Collier (*1*) that differences in the specific gravity of the juice of cornstalks, and of sorghum as well, are mainly attributable to differences in the sugar content of the juice. From these considerations it appeared likely that a comparative examination of the sugar content of sound and of diseased stalks might yield some information on the mode of nutrition of the corn-smut fungus.

MATERIALS AND METHODS

Field corn for this purpose was grown on the Arlington Experiment Farm, through the courtesy of C. H. Kyle, agronomist in Corn Investigations. Material for the analyses was collected between September 11 and September 18, 1925. During that week the plants matured rapidly, so that the kernels were in the "hard-dough" stage at the final cutting. For diseased material, stalks were selected which had one or more smut galls of different sizes at the nodes. Inasmuch as corn plants of a single variety growing in the same field may differ greatly in sugar content, a healthy control plant similar in size, vigor, number of internodes, and state of maturity was selected with each diseased plant. The control plants were taken from hills adjacent to the corresponding diseased plants.

The selected stalks constituting each pair were taken to the laboratory together, and handled as quickly as possible. From the diseased stalk an internode adjoining a smut gall, or in the case of a heavily smutted stalk, an internode between two galls was selected, wiped clean with a damp cloth, and cut into slices about 1 mm. thick with a rotary slicer. Fifty grams of the material were weighed out and preserved in 95 per cent alcohol, with the addition of 0.5 gm. of calcium carbonate. The corresponding internode of the control plant was then similarly sampled.

For the determination of dry matter and sugars the individual samples were handled in the following manner. The alcohol in which the sample had been preserved was poured off through a

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 2
Jan. 15, 1927
Key No. G-556

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weighed filter into a 1,000-ml. flask. The solid residue was transferred to a beaker and extracted five times by boiling for 10-minute periods in 70 per cent alcohol. After each boiling the extract was decanted through the filter into the flask. After the last decantation, the weighed filter with the solid residue was transferred to a tared aluminum weighing can. The weight of the dry residue was obtained by evaporating the free alcohol at 65° C. and drying the residue to constant weight at 105°. For the determination of solids in the alcoholic extract an aliquot was evaporated at a low temperature in a can containing sand, and dried to constant weight in a vacuum oven at 70°. Reducing sugar and total sugar were determined in another aliquot of the alcoholic extract which had been freed from alcohol by evaporation, cleared with neutral lead acetate, and freed from lead by means of sodium oxalate. The reductions were carried out with strict observance of the details of manipulation described by Munson and Walker (6). The cuprous oxide was weighed as such.

TABLE 1.—*Sugar content of smutted and of healthy cornstalks*

Pair No.	Date of cutting	Inter-node (num-beied from base of stalk)	Percentage of dry matter		Reducing sugar as glucose		Sucrose		Total sugar as glucose	
			Healthy plant	Smutted plant	Healthy plant	Smutted plant	Healthy plant	Smutted plant	Healthy plant	Smutted plant
			Heavy infection							
	1925				*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Sept. 11	{ 7	20 66	17.48	13 7	11 9	22.1	13.0	37.0	25 6
		{ 3	17 78	14 17	17 6	15.7	15.7	5.7	34.1	21.7
2	Sept. 14	5	20.76	17 13	15.9	12 6	14.8	4.3	31.5	17.1
3	Sept. 11	8	22 16	18 01	14.3	11 4	19.5	10.5	34.8	22.4
4	do	3	19.00	16.23	16 2	15.5	17 6	5.6	34 7	21.4
5	Sept 18	{ 8	20.37	21 21	9 3	6 7	26.7	10 0	37.4	17.2
		{ 3	17.11	14 54	11 5	2 1	22 8	3.7	35 5	6.0
			Medium infection							
6	Sept. 10	5	19 16	16 69	12 4	10.3	10 7	8.7	23 7	19.5
7	Sept 14	7	17.91	17.35	15 1	12.8	5 5	2 4	20 9	15.3
8	do	8	17.47	17.77	16 0	14.1	7.8	10 8	24.2	25.5
9	Sept. 17	5	17.51	19 51	9.5	8.3	7 6	6.6	17.5	15.2
10	do	5	18 78	16.92	17 5	9 9	7 6	1.9	25.5	11.4
11	Sept. 14	7	15 68	16.31	18 2	12 0	6 4	9.0	24.6	21.5
			Slight infection							
12	Sept 10	4	24.01	26 04	12.5	12 7	24 0	19.8	37.8	33 5
13	Sept 17	5	22.79	22 78	14 1	12.1	13 8	9.9	28.6	22.5
14	Sept 14	7	19.21	17.49	15 8	16.7	13.9	4 8	30.4	21.8

RESULTS

The results of the determinations are given in Table 1, in which the sugar content of the stalks is expressed in percentage of dry matter. The data are presented in three groups, according to the degree of infection of the diseased stalks. The diseased plants of the first group, under "heavy infection" each had three or more large galls

on the stalks. Those of the third group, under "slight infection," had one or two small galls only. Those of the second group, under "medium infection," had one or two fairly large galls and usually one or two small ones, so that in the writers' estimation they represent a degree of infection intermediate between that of the other two groups.

It appears from these data that the growth of *Ustilago zeae* on the stalks of corn results in a considerable reduction of sucrose in the diseased stalks, and an evident, though lesser, reduction of hexoses, the loss of sucrose being greatest in the most severely infected stalks. There are several exceptions among the less heavily smutted plants. Owing to the variability of the sugar content of normal plants, discrepancies of this kind may be expected in view of the difficulty of pairing plants by their appearance. This variability is sufficient to render detection of the effect of slight infections uncertain.

DISCUSSION

While the mode of nutrition of saprophytic fungi and of facultative parasites has been extensively investigated, our knowledge of the nutrition of obligate parasites is still comparatively meager. Incidental observations which may be regarded as having a bearing on the question are found in the literature, although they are not always interpreted from this point of view. Thus, the accumulation of starch in association with rust pustules and in tissues hypertrophied as a result of rust infection has frequently been noted.

Perhaps the earliest observation indicating definitely the utilization of starch by an obligate parasite is that of Reess (10), who states that the starch accumulated in the leaves of *Pinus picea* [*Picea excelsa*] as a result of infection by *Chrysomyxa abietis* is consumed by the fungus at the time of spore formation. The disappearance of starch from tissues infected by different rust fungi was noted also by Peglion (7), Robinson (11), and Mains (4).

The observation, frequently made, that rusts develop less vigorously on shaded plants than on plants growing in full light has been considered as showing a nutritive relation between the products of photosynthesis of the host and the growth of the parasite. Direct experimental evidence of the dependence of rusts upon the carbohydrates of their hosts or upon intermediate products of photosynthesis was first furnished by the work of Ward (13), who showed that the hyphae of *Puccinia glumarum* soon starve and degenerate in leaves of wheat maintained in an atmosphere free from carbon dioxide. Later Fromme (2) found that *P. coronifera* does not develop on its host in the absence of light. The most conclusive evidence of this relation is furnished by the extensive experiments of Mains (4), who showed that in the absence of light or of carbon dioxide the development of *P. coronata* and *P. sorghi* on their hosts is retarded or stopped on account of lack of carbohydrates. If, however, carbohydrates be supplied by means of sugar solutions, *P. sorghi* develops in the dark on seedlings of maize deprived of their endosperms and also on pieces of corn leaves floating on the solutions. Other observations showing the dependence of rusts on the carbohydrate supply of the host have been reported by

Waters (*14*) for *Uromyces appendiculatus* and by Peltier (*8, p. 51; 9, p. 8–11*) for *P. graminis tritici.*

Also of interest is the work of Tischler (*12*) who, in his extended study of the influence of *Uromyces pisi* on *Euphorbia cyparissias,* found that although the hyphae of the fungus penetrate the meristematic tissue of the growing point which contains no sugar, haustoria are formed only in the older vacuolate cells which are rich in starch and sugar. While Tischler interprets this observation in the sense of Miyoshi's (*5*) experiments, it seems that the formation of haustoria in the tissues rich in sugar may be regarded as an indication of the nutritive relations of the fungus.

The writers' data show that the growth of *Ustilago zeae* on cornstalks results in a large reduction of the sugar content of the tissues. It seems evident, therefore, that this fungus also depends on the carbohydrates of the host for a part of its nutriment.

The data do not permit any definite conclusions as to the form in which the carbohydrates are absorbed by the fungus, although they suggest some possibilities. The great difference in sucrose between the healthy plants and the smutted plants might be interpreted as indicating that the fungus uses this sugar primarily. However, the low sucrose content of the infected plants might come about through utilization of the hexoses only. In that case the low sucrose content of diseased plants may be explained on the assumption that the formation of sucrose is hindered by the withdrawal of hexoses, or that sucrose undergoes inversion when the removal of the hexoses disturbs the equilibrium between sucrose and hexoses in the cells.

In the case of rusts it has been suggested that the fungus absorbs intermediate products which occur in the process of carbohydrate formation. This possibility would seem to be excluded in the case of *Ustilago zeae,* since the fungus can invade the young kernels, in which, so far as we know, primary synthesis of carbohydrates does not take place, but to which the carbohydrates are transported as such.

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O

STUDIES ON TOMATO WILT CAUSED BY FUSARIUM LYCOPERSICI SACC.[1]

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INTRODUCTION

That the tomato-wilt disease caused by *Fusarium lycopersici* Sacc. is becoming more important each year to the tomato-growing industry is evidenced by the reports which have appeared in the "Plant Disease Reporter" for the past several years. Not only is the disease of increasing importance in sections where it has already become established, but it is gradually spreading to new localities. It has been present in the Chautauqua region of New York since 1915,[3] was reported as being rather general in its distribution but causing only slight damage in 1921 (*13, p. 319*),[4] and as causing an estimated damage of 2.5 per cent in 1922 (*10, p. 56*). Minnesota reported the disease present for the first time in 1923, a damage of 10 to 20 per cent having been noted in one field (*58*). While primarily a disease of the warmer regions of the country, especially in the Mississippi and Ohio River valleys, it is of increasing economic importance in the cooler tomato-growing regions of New York, and probably of other northern tomato-growing States. Judging from the reports from Minnesota in 1923, the disease may reach serious proportions even in the cooler sections of the country, especially during seasons when high temperatures are prevalent.

There are at present several varieties of tomatoes, either selections of resistant genotypes from standard varieties, or new varieties acquired through hybridization, which are very resistant to the wilt disease. Notable among these are the Marvel, Norton, Norduke, Louisiana Red, and Louisiana Pink varieties. There are many other selections or hybrids now obtainable from the various experiment stations where tomato wilt has been under investigation, which appear to be more or less resistant. These resistant varieties have been distributed from State to State and, in the majority of cases, have retained their resistance under the conditions of the new environment. This, however, has not always been the case.

Journal of Agricultural Research,
Washington, D. C.

34940—27——1   (197)

Vol. 34, No. 3
Feb. 1, 1927
Key No. Kans.-48

The writer has observed that some strains or selections of tomato varieties, resistant in other States, have not proved resistant in Kansas (*60*). A striking example was noted with two lines of Bonny Best, selected for resistance in Ohio, which proved extremely susceptible when grown in Kansas. The Tennessee Beauty, selected by Essary for resistance in Tennessee, proved to be resistant in Ohio (*29*), showed intermediate resistance in California (*50*), but proved to be very susceptible in Kansas. Other cases similar to these are on record.

The cause or causes of this apparent "loss" of resistance may be: (1) Environmental changes which affect either the host or the pathogene or both and introduce factors which modify the character of resistance, or (2) the presence of distinct physiological races of the pathogene in different localities.

REVIEW OF LITERATURE

McClintock (*35*) called attention to the fact already mentioned that strains of tomatoes resistant to the wilt disease in one State or locality are not always resistant in other States or localities.

Shapovalov and Lesley (*50*) recently reported that "Possibly then a few varieties, or perhaps more precisely selections, from other States fail to develop their wilt resistant qualities under California conditions." Clayton (*11*) likewise noted variations in the pathogenicity of strains of *Fusarium lycopersici* isolated from Ohio, Tennessee, Maryland, and Indiana. Edgerton (*15*) stated that different strains of the tomato-wilt fungus did not show the same virulence, or pathogenicity, when tested in the greenhouse.

Leach (*33*) was unable to distinguish between three strains of *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. by means of the relation between temperature and growth or hydrogen-ion concentration and growth, but he found a wide difference between one strain and the other two when growing on a liquid medium containing various sources of carbon.

Hursh (*30*) has demonstrated that urediniospore germination of two biologic forms of *Puccinia graminis tritici* differed considerably in their response to temperature and to hydrogen-ion concentrations. The form more restricted in its host range was also found to be more limited in tolerance of extremes of hydrogen-ion concentration and of temperature as determined by urediniospore germination. He concludes that "at least some biologic forms apparently possess individual physiologic characteristics demonstrable by physical and chemical studies, and these characteristics alone may be sufficient to establish them as definite taxonomic entities."

Matsumoto (*37, 38*) found that different isolations of *Rhizoctonia solani* varied physiologically. LaRue and Bartlett (*31*), working with *Pestalozzia guepini*, demonstrated that by precise methods the species could be broken up into an indefinite number of strains, the number depending only upon the precision of their methods. Harter and Weimer (*25*), studying 18 strains of *Rhizopus nigricans* from different parts of the United States and Europe, all equally parasitic on sweet potatoes, were able to separate one strain from the others by means of a study of their temperature relations. One strain was found to have a lower optimum and maximum temperature for growth than the other 17.

SCOPE OF INVESTIGATION

It is not improbable that the different degrees of pathogenicity exhibited by various strains of an organism are correlated with some physiological difference or differences among them. Working on this assumption, the relations of temperature and of hydrogen-ion concentration to growth have been studied in 24 strains [5] of Fusarium isolated from wilted tomato plants received from various parts of the country. An attempt has been made to correlate the results of these studies with the different degrees of pathogenicity that the strains exhibited in the greenhouse tests. The characteristics of the strains on various media are recorded in this paper. Studies have been made of carbon sources, the method of utilization of the carbon source, and the production and isolation of toxic substances produced as a result of growth upon synthetic media.

SOURCE OF CULTURES

The cultures used in these experiments are listed below. The numbers are those under which the several isolations are entered in the collection of the department of botany and plant pathology of the Kansas State Agricultural College.

In the work reported here the various strains will be referred to by number only, unless it is deemed expedient to do otherwise.

Acces-sion No	Locality in which found	Date isolated	Acces-sion No.	Locality in which found	Date isolated
60	Cherryvale, Kans	June 19, 1922	129	Missouri	Received in 1923.
61	Hutchinson, Kans	June 23, 1922	130	Arkansas	Do
68	Manhattan, Kans	July 17, 1922.	131	New York	Do.
119	Ames, Iowa	Received in 1923	132	Ohio	Do.
120	Texas	Do.	133	Manhattan, Kans	Sept 8, 1923.
121	Tennessee	Do	134	St. John, Kans	Aug. 17, 1923
122	Louisiana No. 399	Do.	143	Tennessee	Received in 1923.
123	Virginia	Do.	144	Wisconsin	Do
124	Louisiana No 57	Do	145	Ohio	Do.
125	Louisiana	Do	149	Georgia	Isolated in 1917.
126	New Jersey	Do.	150		Subculture of Wollenweber's type.
127	Louisiana No 397	Do.			
128	New Jersey	Do.			

PATHOGENICITY OF THE STRAINS •

All the strains of Fusarium which were used in this work were pure lined by the usual methods. All were tested in the greenhouse to prove their pathogenicity. That some strains are more virulent and more destructive than others in their attack upon seedling tomatoes is clearly evidenced by the behavior of seedlings when grown in the greenhouse in sterilized and in artificially inoculated soil.

The soil used was sandy loam sterilized for 60 minutes in an autoclave at 15 pounds pressure. After sterilizing, it was inoculated by mixing with each 4-inch pot of soil approximately 50 gm. of of a 3-weeks-old wheat-kernel culture of the organism. Seed of the Bolgiano Red tomato was sown immediately in each pot. The plants

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[5] By "strains" as used in this paper is meant merely separate isolations from different localities in the United States. The term does not imply physiological races, unless so stated.

were kept in a greenhouse in which the temperature was not constant, the fluctuation during these experiments being between 75 and 85° F.

At the end of four weeks symptoms of wilt became evident on plants growing in soil inoculated with cultures 122, 127, 131 and 144. At the end of six weeks these were badly wilted and No. 120 was beginning to show some wilting. At the end of eight weeks all the plants in each pot were counted, cut at the surface of the ground with a sharp scalpel, and examined for symptoms of fibrovascular browning. At this time the plants growing in soil inoculated with strains 122 and 127 were completely dead, and those in soil inoculated with strains 131 and 144 were practically dead. The plants in soil inoculated with strain 120 were very badly wilted and a few plants in this pot were also dead. The remainder of the plants showed only a slight wilting and a large number of yellowed leaves. Yellowed leaves, however, in this particular experiment can not be taken as a symptom of wilt, since control plants, growing in sterile soil, showed some yellowed leaves at the end of eight weeks caused by crowding in the pot. In Table 1 are given the percentages of seedlings showing fibrovascular browning at the end of eight weeks. The germination of the seed used was very uneven, and different numbers of plants were therefore obtained in the several pots.

TABLE 1.—*Percentage of seedlings of Bolgiano Red tomatoes showing fibrovascular browning after eight weeks growth in infested soil*

Strain No.	Total number of plants	Number showing browning	Per cent diseased	Strain No.	Total number of plants	Number showing browning	Per cent diseased
60	10	6	60	129	29	3	10
61	25	2	8	130	25	5	20
68	17	8	47	131	19	17	89
119	24	6	25	132	19	1	5
120	31	18	58	133	32	6	19
121	17	15	88	134	7	2	29
122	20	20	[a] 100	143	28	2	7
123	26	12	46	144	17	14	82
124	15	3	20	145	9	0	0
125	34	5	15	149	15	14	93
126	32	15	47	150	24	20	83
127	17	17	[a] 100	Control	55	0	0
128	26	17	65				

[a] Tops dead and dry.

It is evident from Table 1 that all the strains of this organism do not attack seedling tomato plants with the same virulence. Strains 120, 122, 127, 131, and 144 are outstanding in that they produced wilt before any of the other strains and the plants infected were more severely wilted than the plants infected with other strains. Strains 121, 149, and 150 were exceptional in that while the plants were badly infected, as evidenced by the high percentage of fibrovascular browning, they showed no wilting. Strain 145, a strain isolated in Ohio and received from C. D. Sherbakoff, failed to cause any fibrovascular browning or wilting. However, it was similar to pathogenic strains in all cultural habits, as far as these were studied. As a result of these pathogenicity tests, strains 120, 122, 127, 131, and 144 are placed together as a group and designated as the 120 group. All other strains are arbitrarily placed in a second group designated as the 60 group.

CULTURAL CHARACTERISTICS OF THE STRAINS

The strains placed in the 120-group differ from the strains in the 60-group in several ways. They produced only a slight pink color, on acidified potato-dextrose agar. All the other strains produced a deep-vinaceous color on this agar, typical of Fusaria in the Elegans section as described by Sherbakoff (*51*) (pl. 1). Furthermore, on potato agar strains in the 120 group produced an abundance of microconidia in 10 days, giving the culture a powdery appearance, while all the other strains produced no spores of any kind in that length of time on the same medium. Another constant difference between the 120 group and the 60 group was observed when the strains were grown on sterilized wheat kernels. The 120 group produced an abundance of macroconidia in sporodochia, giving to the kernels a faint pink to a deep vinaceous-red tinge. All the other strains, when grown on sterilized wheat kernels, produced few macroconidia but an abundance of microconidia. They also produced a purplish vinaceous color of varying intensity. On steamed rice, the 120 group produced a purplish vinaceous color but less intense than that produced by the 60 group.

TABLE 2.—*Notes on 14-months-old cultures of Fusarium lycopersici growing on potato-dextrose agar in test tubes 380 cm. long (notes taken June 29, 1925)*

[Plus sign present; minus sign absent]

Culture No.	Zonation [a]	Color [b]	Habit of growth of aerial mycelium	Sclerotia [c]	Macroconidia	Microconidia [d]	Chlamydospores [e]	Remarks
60	Very slight	None	Very slight	−	−	−	+	
61	___do	___do	___do	−	−	−	+	
68	Slight	Faint	Absent	−	+	+	+	Macroconidia v e r y scarce.
119	Very definite	Medium	___do	−	−	+	+	
120	None	None	Rather abundant	+	−	+	+	
121	Slight	Faint	Absent	−	−	+	+	
122	None	None	Medium	+	−	+	+	Sclerotia rare.
123	Very definite	Slight	___do	−	−	+	+	
124	None	None	___do	−	−	−	+	Chlamydospores very few.
125	Irregular	Medium	Slight	−	−	+	+	Microconidia scarce.
126	Slight	Faint	Absent	−	−	+	+	
127	None	None	Moderate	+	−	+	+	Microconidia v e r y abundant.
128	Definite	Medium	Absent	−	−	+	+	
129	Very definite	Deep	___do	−	−	+	+	
130	None	None	Moderate	−	+	+	+	Chlamydospores and macroconidia scarce.
131	___do	___do	Abundant	+	−	+	+	Sclerotia rare.
132	___do	___do	Very slight	−	−	−	−	
133	Slight	Faint	Absent	−	−	+	+	
134	None	None	Slight	−	−	+	+	
143	Slight	Faint	Absent	−	−	+	−	
144	None	None	Abundant	+	−	+	+	Microconidia v e r y abundant.
145	___do	Very faint	Absent	−	−	+	+	
149	Faint	Faint	Abundant	−	−	+	+	
150	Definite	Medium	Slight	−	−	+	+	Chlamydospores very abundant.

[a] Bands of color usually delimited sharply; No. 125 only roughly zonate.
[b] Color typical of Elegans section when present; varying degrees of vinaceous purple.
[c] Sclerotia small and colorless.
[d] Microconidia oval continuous or 1-septate.
[e] Chlamydospores smooth, thick walled, colorless to brown, terminal or intercalary, often in pairs or in chains.

The strains were grown on potato-dextrose agar in tubes 380 cm. long with a 20 mm. diameter, in the dark at room temperature. Macroscopic and microscopic examinations of the cultures were made at the end of 14 months, and the results of these are given in Table 2. Wide variations were noted between the different strains. For example, they varied from nonzonate to very definitely zonate, from colorless to deep vinaceous purple, and from prostrate vegetative growth to an abundant production of aerial mycelium. Strains in the 120 group were alike in the lack of zonation, lack of color production, and the presence of abundant aerial mycelium. These characters were not, however, confined to these five strains, and so they can not be distinguished from the remaining strains by macroscopic characters alone. The production of sclerotia, however, was confined to these five strains, no other strain ever producing them during the course of these investigations. The objection might be justly raised that these cultures, by the lack of macroconidial production, were not cultures approaching the noim. Furthermore, they may have varied from the norm in different degrees. Repeated systematic attempts have been made to secure these cultures in "Hochkultur" over a period of four years without satisfactory results.

At the higher temperatures in culture on potato-dextrose agar, the different strains varied more in growth habits than at temperatures approaching the optimum. This is shown by Plates 2 to 7, inclusive, which are photographs of the strains after six days' growth on potato-dextrose hard agar at 32°C. It should be noted here that strains 120, 122, 127, 131, and 144 appear white in the plate, but in reality they were very faintly tinged with pink. This pink tinge was always present in thalli of these strains on this agar at all temperatures, when grown in culture dishes. Strains 61 and 134, like the five strains mentioned above, showed only a slight color. The higher temperatures were conducive to the production of aerial mycelium, while the lower temperatures were conducive to the production of color. Strains 61 and 134, although evidently lacking the deep purplish vinaceous color typical of the Elegans section of Fusarium at 32° C., developed it at temperatures of 24° and below. However, in common with strains 60, 129, and 132, they never developed such a deep color as did the other strains included in this group.

Strains 144 and 150 were selected as representative of the two groups and were grown on four agars suggested by Sherbakoff (*51*) to give normal cultures. The observations made on these cultures are presented in Table 3. The fact that strain 150 was not observed in a good sporulating condition on any of these media and that strain 144 produced macroconidia in abundance only upon oatmeal agar, makes a comparison of the two strains somewhat unsatisfactory. The data are presented, however, in conjunction with other data upon the strains to indicate that variations may be expected when studying different isolations of organisms causing tomato wilt.

TABLE 3.—*Spore measurements of 6-weeks-old cultures of strains 144 and 150 grown on different agars*

Agar used	Measurements of strain 144 (microns)	Measurements of strain 150 (microns)

Rice	Microconidia:
	  Maximum 17.0.
	  Minimum 5.1.
	  Average 9.55 (100).[a]
	Macroconidia, none.
	Chlamydospores·
	  Maximum 14.0.
	  Minimum 7.22
	  Average 9.77 (40).
		Microconidia:
		  Maximum 15.3.
		  Minimum 6.8.
		  Average 10.91 (50).
		Microconidia, none.
		Chlamydospores:
		  Maximum 12.75.
		  Minimum 6.80.
		  Average 9.21 (40).

Lima bean	Microconidia:
	  Maximum 17.0.
	  Minimum 3.4.
	  Average 8 87 (50).
	Chlamydospores.
	  Maximum 13.6×8.5
	  Minimum 6.8×6.8.
	  Average 9.82×8.02 (25).
	Macroconidia:
	  Maximum 34.0.
	  Minimum 11.9.
	  Average 21.28 (25).
		Microconidia:
		  Maximum 17.0
		  Minimum 5.1.
		  Average 9.69 (50).
		Chlamydospores:
		  Maximum 11.9×8.5.
		  Minimum 5.95×5.52.
		  Average 8.17×7.07 (25).
		Macroconidia, none.

Wheat	Microconidia.
	  Maximum 20.4.
	  Minimum 3.4.
	  Average 8.46 (50).
	Chlamydospores·
	  Maximum 11.9.
	  Minimum 6.8.
	  Average 9.77 (25).
		Microconidia:
		  Maximum 15.3.
		  Minimum 5.1.
		  Average 8.87 (50).
		Chlamydospores:
		  Maximum 10.2×5.1.
		  Minimum 5.1×5.1.
		  Average 7.70 (25).

Oatmeal	Microconidia:
	  Maximum 13.6.
	  Minimum 3.4.
	  Average 7.31 (50).
	Chlamydospores:
	  Maximum 11.9.
	  Minimum 6.8.
	  Average 9.19 (50).
	Macroconidia (sporodochia):
	  Maximum 51.0
	  Minimum 18.7.
	  Average 39.27 (200).
		Microconidia.
		  Maximum 13.6.
		  Minimum 6 8
		  Average 9.31 (50).
		Chlamydospores:
		  Maximum 10.62.
		  Minimum 6.8.
		  Average 8.50 (50).
		Macroconidia, none.

[a] Numbers in parentheses indicate number of spores measured from which the averages were obtained.

PHYSIOLOGICAL STUDIES ON FUSARIUM LYCOPERSICI

TEMPERATURE AS RELATED TO GROWTH

A great deal of work has been done upon the temperature relationships of pathogenic organisms and the diseases which they cause. No attempt will be made here to review the great mass of literature that has accumulated upon this subject, except where pertinent to this study.

The object of studying the temperature relationships of a number of strains of Fusarium which had been isolated from wilted tomato plants in various parts of the country was to determine if by such a study constant physiological differences between strains could be discovered. Brown (8), speaking of the problems that may be solved by a study of temperature relations, states that one of these is the problem of physiologic races. "Accepting the existence of several strains of the same organism, what physiological differences can one establish between them, and can one correlate with these any of the distinct appearances of the particular strains?"

Edgerton and Moreland (17), working with a Louisiana strain of *Fusarium lycopersici* on bean-pod agar, determined the optimum temperature for growth over a five-day period to be 29° C. Clayton (11),

working with an Indiana strain on potato hard agar, determined the optimum for growth to be 28°. It is evident that this organism is one requiring relatively high temperatures for maximum growth over a short period of time when grown on artificial substrata. That this relation may not hold for growth in soil over longer periods of time is possible since Balls (*3*) found that the optimum temperature for growth of a fungus is a variable factor, depending on the physiological history of the fungus as well as upon the immediate conditions under which it is growing. In this connection, however, Clayton (*11*) has demonstrated that the optimum soil temperature for the disease is approximately 28°, while at temperatures above 33° and below 21° it is practically inhibited. That the substrate upon which the organism is growing is one of the most important of the factors that influence growth is probable. Brooks and Cooley (*7*), studying the temperature relations of several apple-rotting fungi under different conditions, concluded that the temperature response of a particular organism was modified by the medium upon which it was grown. The minimum temperature for the various fungi studied varied greatly with the food material, but there was little variation in the optimum under the same conditions. Link (*34*) found in a study of Fusaria related to potato tuber rot that the optimum and maximum temperature points varied with the medium used. His results were corroborated by Goss (*24*), who stated as a result of his studies upon the temperature and humidity relations of some Fusaria rots of the Irish potato, that the cardinal points may vary with the medium used. It might be expected, therefore, that the strains of the same organism from widely different sections of the country, representing the northern and southern limits of its geographical range, would differ in their physiological behavior when brought together and subjected to the same conditions.

Fawcett (*19*) has summed up the conditions that influence the rate of growth of a fungus in culture and pointed out that if one of these is to be studied the others should be definitely stated. The conditions that should be given are the nature of the fungus, the nature of the medium, temperature, radiation, and duration.

Experimental Methods

The medium used for this work was hard potato-dextrose agar, made according to the formula of Hopkins (*27*).[6] The medium was prepared as needed.

The temperature desired was maintained in a low temperature incubator. Constant temperatures were used, and a fluctuation of not more than 0.5° from the temperature desired was recorded at any time. The cultures were kept in the dark at all times, being removed only for daily measurements. Fawcett (*19*) had previously shown that the removal of cultures for a brief period of time each day for measurements does not influence the growth rate. The duration factor was investigated before the final experiments were made. That this factor is of importance in measuring physiological processes has been recorded recently in a number of papers (*3*, *8*, *19*, *47*, *48*).

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[6] Two hundred gm. of cubed potatoes brough, to a boil in 1,000 c. c. of tap water. To the strained potato broth was added 10 gm. of dextrose and 20 gm of agar.

The duration of the time interval chosen for this work was four days. Growth was allowed to take place for two days before experimental measurements were taken. It was found that with the temperatures and fungi used the time interval or the relation of this interval to the entire culture period had little significance. The same results would have been obtained if the measurements of all the days in the culture period had been averaged rather than those chosen, as will be brought out later in this paper.

From the stock cultures of the strains of the organism subcultures were made in Petri dishes. These were held for three days at the temperature which was to be used for the experimental cultures except at the lower temperatures, where five days were used. A small piece of agar with mycelium from just back of the growing margin of the colony was transferred from these subcultures to the experimental dishes, which were then inverted and placed in the culture chamber of the incubator. With temperatures below 28° C. no difficulty was experienced in keeping the humidity of the chamber at a point which prevented drying of the medium. With temperatures above 28° water was atomized into the chamber three times a day. A relative humidity of approximately 85 per cent was maintained in the culture chamber by observing these precautions. Humidities were measured by means of a wet-bulb apparatus. Gradual drying of the medium, it was found, would decrease the growth rate of the organism, and the colony would then have the appearance of a staled colony, as described by Brown (*8*). This decrease in growth rate may have been due to a deficiency of moisture in the medium or to a decrease in the diffusion of food materials through the more concentrated substrate.

In taking the measurements the diameter of the colony was measured at the same hour on consecutive days. The advantages of this method of measuring growth over the dry-weight method have been pointed out by Brown (*8*).

In getting the weekly growth rates, tubes 380 cm. long with a 20 mm. inside diameter were used. A piece of cork was cemented in the open end of the tubes about 60 mm. from the mouth. Hard potato-dextrose agar was poured in and the tubes plugged and sterilized. They were then placed in a horizontal position until the agar had solidified, resulting in an even layer of agar the entire length of the tube. The agar in the tubes was inoculated with the strains and placed in a horizontal position. The tubes were kept at room temperature in the dark, the maximum and minimum for each 24-hour period being recorded. Weekly increases in growth were also recorded.

EXPERIMENTAL RESULTS

A summary of the results of the measurements of daily growth rates of the organisms studied appears in Table 4. Two diameters of each colony were measured at the end of each 24-hour period. The average of these two measurements was taken to represent the average diameter of the colony at the time of measurement. The average of all the cultures used in each temperature series was computed and these averages appear in Table 4.

TABLE 4.—*Mean average of the daily growth rates (in millimeters) of 24 strains of Fusarium lycopersici at temperatures ranging from 10° to 35° C.*

Strain No.	Mean average of daily growth rate (mm.) at temperatures of—						
	10° C	12° C.	15° C.	16° C.	18° C.	20° C.	22° C.
60	[a]0.87 (3)	1 64 (2)	3.08 (3)	3 66 (4)	3.20 (2)	4 84 (5)	3.50 (2)
61	1.19 (3)	.80 (2)	3.13 (3)	3.80 (4)	4 31 (2)	5.57 (4)	6.00 (2)
68	.56 (3)	1.03 (2)	2.12 (3)	2 97 (4)	2.81 (2)	4 25 (5)	4.58 (2)
119	1 04 (3)	1.28 (2)	2.37 (3)	3 00 (2)	3.31 (2)	4 99 (5)	5.00 (2)
120	1.29 (3)	1 36 (2)	2.69 (3)	3.59 (5)	4.36 (2)	6.08 (10)	4.70 (4)
121	95 (3)	1.57 (2)	2.93 (3)	3 81 (3)	3.53 (2)	5.15 (5)	4.41 (2)
122	95 (3)	1 50 (2)	2.31 (3)	2 63 (4)	3.90 (2)	5 54 (5)	5 16 (2)
123	1.10 (3)	1.50 (2)	2.48 (3)	3 43 (4)	3.65 (2)	5.60 (5)	4.83 (2)
124	1 09 (2)	1.41 (2)	2.39 (3)	3 09 (3)	3.69 (2)	4 53 (5)	5.00 (2)
125	1 17 (3)	1 46 (2)	2 28 (3)	3.81 (4)	3.75 (2)	5.82 (5)	5 83 (2)
126	1.01 (3)	1 21 (2)	2.36 (3)	3.13 (4)	4 04 (2)	4.86 (5)	6.00 (2)
127	1 07 (3)	1 28 (2)	2.40 (3)	3 61 (4)	4.21 (2)	5.69 (5)	5.66 (2)
128	1.01 (3)	1 13 (2)	2.32 (3)	2.84 (4)	3.70 (2)	5.16 (5)	5.25 (2)
129	1.37 (3)	1 43 (2)	2.66 (3)	3.39 (4)	3 69 (2)	5.41 (5)	6.25 (2)
130	1 15 (3)	1 34 (2)	2.57 (3)	2 76 (4)	3.67 (2)	5.11 (5)	5.75 (2)
131	1.08 (3)	1 23 (2)	3.30 (3)	3.95 (4)	4.34 (2)	6.27 (5)	5.25 (2)
132	67 (3)	1 25 (2)	2.29 (3)	2.87 (4)	2 84 (2)	3 99 (5)	6.50 (2)
133	.79 (3)	1.27 (2)	2.65 (3)	3 09 (4)	3 65 (2)	5.18 (5)	5.33 (2)
134	.72 (3)	1.48 (2)	2 27 (3)	3.63 (4)	3.73 (2)	5.17 (5)	4.16 (2)
143	1.33 (3)	.98 (2)	2 62 (3)	3.52 (4)	3 67 (2)	5.40 (5)	--------
144	.94 (3)	1.39 (2)	3 05 (3)	4 11 (4)	4 15 (2)	6.13 (5)	--------
145	1.08 (3)	1.28 (2)	2 60 (3)	3 13 (4)	2.96 (2)	5.05 (5)	--------
149	.68 (3)	1 30 (2)	1.95 (3)	3.07 (4)	--------	2.57 (2)	--------
150	1 11 (3)	.86 (2)	2.13 (3)	2.94 (4)	--------	2.67 (2)	--------

Strain No.	Mean average of daily growth rate (mm.) at temperatures of—						
	24° C.	25° C.	26° C	28° C	30° C	32° C	34° C.	35° C.
60	6.38 (3)	6.79 (3)	6.78 (4)	7 25 (2)	6 75 (4)	3.08 (2)	0 68 (3)	(b)
61	8.94 (5)	9.10 (3)	7 19 (5)	9.01 (4)	5.78 (4)	3 16 (3)	.99 (3)	(c)
68	7.42 (5)	5.83 (3)	6 21 (5)	5.71 (4)	6.00 (4)	2 37 (4)	.94 (3)	(b)
119	7.28 (5)	7 62 (3)	6 86 (5)	6.83 (4)	6.48 (4)	1.66 (4)	.34 (3)	(b)
120	7.25 (3)	7 75 (3)	7.54 (7)	7.40 (8)	6.71 (8)	3 39 (5)	1.06 (6)	0.28 (3)
121	5.57 (3)	6.22 (3)	6 41 (5)	6 25 (2)	4 93 (4)	2.03 (4)	.56 (3)	(b)
122	7.12 (3)	7 67 (3)	7 92 (4)	6 84 (4)	5.72 (4)	2.36 (3)	.93 (3)	.14 (3)
123	5.20 (3)	5.68 (3)	5.87 (5)	5.80 (4)	5.21 (4)	3 97 (3)	1.06 (3)	(b)
124	7.52 (5)	8.10 (3)	6 91 (6)	6 57 (4)	6 29 (4)	4 25 (3)	.67 (3)	(b)
125	6.28 (3)	7.48 (3)	7 08 (5)	6.66 (4)	6.34 (4)	3.10 (4)	.69 (3)	(b)
126	6.90 (5)	7.37 (3)	7.36 (5)	6 40 (4)	5 74 (4)	3.38 (4)	1.25 (3)	(b)
127	7.00 (5)	7.83 (5)	7.67 (5)	7.34 (4)	6.42 (4)	3 56 (3)	.68 (3)	14 (3)
128	6 06 (4)	8.60 (3)	6.49 (5)	7.02 (4)	6.39 (4)	2 91 (4)	.02 (3)	(b)
129	7.63 (4)	8 58 (3)	8.54 (5)	7.96 (4)	8.32 (4)	4.97 (4)	1.12 (3)	57 (3)
130	7.11 (5)	7 23 (3)	7.35 (4)	6.40 (6)	6.44 (4)	3 08 (4)	.34 (3)	(b)
131	8.62 (2)	8.41 (3)	7 30 (5)	7.53 (4)	6.90 (4)	3.20 (3)	1.68 (3)	(b)
132	7.24 (5)	8.02 (3)	6 59 (5)	7.59 (5)	7.01 (4)	3.55 (4)	.37 (3)	(b)
133	6.88 (5)	6.98 (3)	6.20 (5)	6.40 (5)	5 24 (4)	3.60 (4)	1.00 (3)	(b)
134	7.37 (2)	6.31 (3)	7.67 (4)	6.80 (4)	5.89 (4)	1.53 (2)	.43 (3)	(b)
143	6 42 (3)	7.37 (3)	7.75 (5)	6.81 (3)	7.15 (2)	3.41 (3)	-- ------	(b)
144	6.88 (3)	8.04 (3)	7.04 (5)	6.65 (3)	5.53 (2)	3 35 (3)	----------	.28 (3)
145	6.44 (3)	6 23 (3)	6.29 (5)	4.66 (3)	4.56 (2)	2.37 (2)	----------	.14 (3)
149	5 93 (3)	7.45 (3)	6.19 (3)	----------	5.51 (2)	2.79 (2)	----------	.14 (3)
150	6.20 (3)	6.54 (3)	4 85 (3)	4.79 (2)	4.65 (2)	1.71 (4)	----------	.21 (3)

[a] Numbers in parentheses indicate number of colonies measured from which the averages were obtained.
[b] Perceptible but not measurable growth.
[c] No growth.

From the data presented in Table 4, the growth curves of the several strains were drawn. These curves appear as Figures 1 and 2. Table 5 presents the average increase in diameter of the two groups of strains over the four-day growth period previously described. In Table 6 the weekly growth rates of the several strains over a six-weeks' period are shown, including the mean weekly temperatures and the average growth rates of the several strains, collectively and individually. Table 7 presents the mean 24-hour diameter increases of a few of the

strains for five-degree temperature intervals from 10 to 30° C. The average of 5, 6, or 7 day growth periods are also given. Owing to slight variations in the size of the inoculum used, it was thought best in this case also to discard the first day's growth of all cultures. The object of presenting these data, analyzed as they are, is to show that with these organisms the length of observation period, within the limits of these experiments, is not of major importance in computing average daily growth rates. In this connection also, the mean weekly temperatures and growth rates of these strains over a six-weeks period, as presented in Table 6, are of interest.
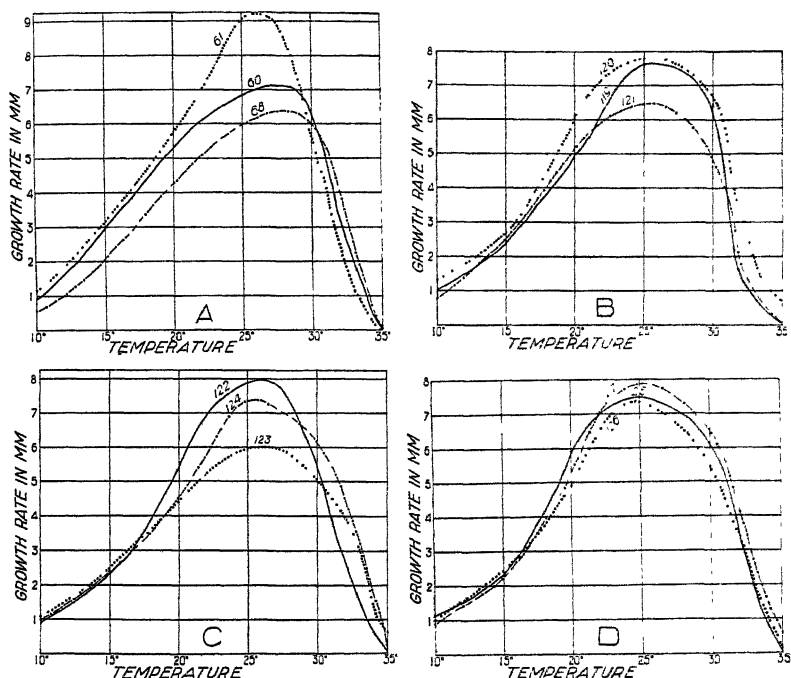
Fig. 1.—Temperature-growth curves (smoothed) of strains of *Fusarium lycopersici* Nos. 60, 61, 68, 119, 120, 121, 122, 123, 124, 125, 126, and 127

TABLE 5.—*Average daily increase in diameter (in millimeters), at different temperatures of the two groups of strains used in temperature studies*

Group	Average daily increase in diameter (mm.) at temperatures of—							
	10° C.	12° C.	15° C.	16° C.	18° C.	20° C.	22° C.	24° C.
120 group	1. 0.	1 35	2. 75	3. 59	4 19	5. 94	ᵃ 5. 19	7. 37
60 group	. 99	27	2 48	3 26	3. 54	4. 81	6. 61	6. 77

	25° C.	26° C.	28° C.	30° C.	32° C.	34° C.	35° C.
120 group	7. 94	7. 49	7. 15	6. 25	3 17	1. 09	0. 32
60 group	7. 18	6. 75	6 60	6 03	2. 99	0. 73	

ᵃ Grown in a separate incubator in cool room,
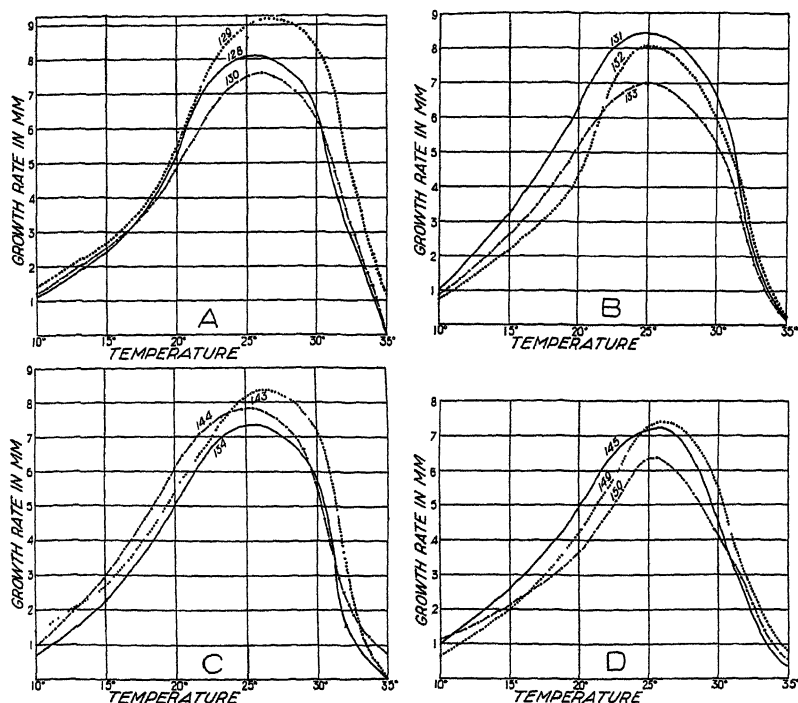
FIG. 2.—Temperature-growth curves (smoothed) of strains of *Fusarium lycopersici* Nos. 128, 129, 130, 131, 132, 133, 134, 143, 144, 145, 149, and 150

TABLE 6.—*Mean weekly temperatures and weekly growth rates of strains of Fusarium lycopersici*

Strain No.	Growth rate in millimeters						
	First week at 75 3° F. mean temperature	Second week at 72° F. mean temperature	Third week at 72 7° F. mean temperature	Fourth week at 76.25° F mean temperature	Fifth week at 70.8° F. mean temperature	Sixth week at 74.6° F. mean temperature	Average
60	43	45	40	47	40	45	43.3
61	37	44	39	44	40	42	41.0
68	40	43	38	42	46	42	41.8
119	56	41	37	41	39	42	42.6
120	37	40	37	36	42	50	40.3
121	44	45	39	42	41	45	42.6
122	38	44	37	41	38	42	40.0
123	35	38	45	28	39	38	37.1
124	67	43	37	43	38	41	44.8
125	42	47	41	47	41	47	44.1
126	39	43	37	41	39	43	40.3
127	38	47	37	42	41	47	42.0
128	41	45	39	44	40	45	42.3
129	46	47	43	49	43	47	45.8
130	37	41	37	43	36	41	39.1
131	42	44	44	47	40	40	42.8
132	38	46	40	43	38	42	41.1
133	40	46	40	46	38	45	42.5
134	51	42	37	42	40	47	43.3
143	39	40	40	46	41	46	43.1
144	43	43	38	41	43	47	42.3
145	39	45	40	51	44	48	44.1
149	40	42	35	51	31	40	39.6
150	30	39	36	40	39	42	37.6
Average	41.7	43.5	38.8	43.2	39.8	43.9	

TABLE 7.—*Mean daily diameter increases in millimeters of mycelial disks of Fusarium strains isolated from wilted tomato plants*

Strain No.	24-hour period No.	Mean diameter increase at—				
		10° C.	15° C.	20° C.	25° C.	30° C.
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
60	1	1.15	4 08	5.75	7.50	7.06
	2	1.00	3.66	5.42	7.50	6.50
	3	1.17	3 00	5.42	6.33	6.75
	4	.58	2.79	5.08	7.00	5.62
	5	.71	2 79	4 92	6.33	6.12
	6	1.21	2 08	5.25	---------	6.00
	7		2.42			
	Average entire period	.97	2.97	5.31	6.93	6.34
	4-day average	.86	3.06	5.21	6.79	6.25
119	1	1.29	2.58	5.08	7.83	5 75
	2	1.23	2.66	5.17	7.75	5.87
	3	1.21	2.33	4 58	6.92	6.50
	4	.87	2.29	6.25	7.83	6.87
	5	.87	2 21	6 08	8.00	7 62
	6	1.33	2.58	5.58	---------	7 25
	7		2.00			
	Average entire period	1 13	2.38	5.45	7.67	6 64
	4-day average	1.04	2.37	5.52	7.62	6.71
120	1	1.58	3.16	7.34	8 83	7 12
	2	1.58	2.58	6.88	8 87	6.50
	3	1.54	2.92	6 79	7.54	6.37
	4	88	2.71	7.71	7 25	6.25
	5	1.16	2.54	6.83	7.33	4 12
	6	1.16	2.87	7.00	---------	5.87
	7		2.12			
	Average entire period	1.31	2.70	7.09	7 96	6.04
	4-day average	1.29	2.69	7.05	7.75	5 81
127	1	.92	3.16	6.00	9.00	3.75
	2	1.00	2.66	5.92	8.33	6.00
	3	1.50	2.33	6.33	7.33	7.25
	4	.58	2.21	5.66	8.33	5.12
	5	1.21	2.42	6.25	7.33	5.00
	6	1.04	2.23	6.33	---------	5.37
	7		2.50			
	Average entire period	1.04	2.50	6.08	8.06	5.41
	4-day average	1 07	2.40	6.04	7.84	5.84
144	1	.88	3.87	7.21	9.33	5.69
	2	1.25	3.37	6.66	9.08	6.62
	3	1.00	3.17	6.66	7.46	6.93
	4	.87	2.83	6.75	8.37	3.94
	5	.63	2.84	6.42	7.25	4.62
	6	.92	2.92	6.58	---------	5.50
	7		2.62			
	Average entire period	.92	3 09	6.71	8.29	5.55
	4-day average	.94	3 09	6.62	8.04	5.53
150	1	1.17	2.63	5.54	6.17	5.31
	2	1.17	2.46	5 00	5.42	4.37
	3	1.37	2.17	4.83	4.92	4.75
	4	1.00	2.33	6.17	5.75	4.50
	5	.92	2.38	5.42	6.08	5.00
	6	1.37	2.16	5.58	---------	3.75
	7		2.13			
	Average entire period	1.16	2.32	5.42	5 67	4.61
	4-day average	1.09	2 33	5.35	5.54	4.65

DISCUSSION OF RESULTS

From the data presented in Table 4 and Figures 1 and 2 it is evident that the temperature for optimum growth over the four-day period under observation varies with the strain, the optima being within the limits of 24 to 28° C. For strain 60 from the southeastern part of Kansas the optimum is 28°, while for strain 131 from western New York the optimum is 24°. The other strains are grouped around 25 and 26° as their optimum growth temperatures.

As stated above, strains in the 120 group were distinctly more virulent in their attack upon seedling tomatoes in the greenhouse than the remaining strains. Certain other characters also differentiated

them from the strains in the other group. By reference to Table 5 it will be noted that at all temperatures strains in the 120 group were more rapid growers than those in the 60 group. Although both groups found their optimum temperature for growth at 25° C., the 120 group had a wider range of growth temperatures than the 60 group. This fact may explain why the strains of this group are found in New York and Wisconsin, as well as in Texas and Louisiana. From the latter State were obtained cultures of the organism which fell in both groups. One culture only was received from New York, one from Wisconsin, and one from Texas, and all fell in the 120 group.

The difference in the growth rates is widest at the optimum point, 25° C., a fact which is brought out in Figure 3. This difference in growth rate can be correlated with other characters; for example, lack of the production of a deep vinaceous red on agar rich in dextrose and the abundant production of macroconidia in sporodochia with a faint pink tinge when grown on wheat kernels.

The temperature curves of the various strains show wide differences. The most outstanding difference is noted in strain 129, isolated from a wilted tomato plant in Missouri by I. T. Scott. This organism grew with distinctly greater rapidity at the higher temperatures than the other strains.
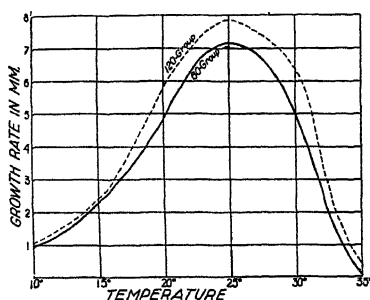
FIG. 3.—Temperature-growth curves of the two groups of strains of *Fusarium lycopersici* used in the temperature studies

Table 6 presents the mean weekly temperatures and the weekly growth rates of the 24 strains used in these studies. As can be readily seen, these fungi, when grown on potato-dextrose agar, are not of the staling type described by Brown (8). No evidence of a retarding influence on growth was noticed at any time during the six weeks in which these cultures were under observation. Fawcett (20, 21) found the same to be true of three fungi with which he worked. Being of the nonstaling type when grown on this medium, they should show, theoretically, no diminution in growth rate at the higher temperatures from day to day. That this was actually the case is shown by the data presented in Table 7. From this table it can be seen that the growth rates at the lower temperatures were very uniform from day to day, but that at the higher temperatures fluctuations took place. These can not be explained at this time. However, while in some cases there is an apparent decrease in growth rate with increase in duration at 30° C., in as many other cases there is an apparent increase. That the duration of the time interval used for obtaining the mean daily growth rates is of little importance in this case is evident from the averages presented in Table 7. With the four-day average taken after the initial two-day growth period is presented the average of the entire culture period of five to seven days. There is little difference between the two averages. With these fungi, therefore, it seems immaterial when determining the daily increases of growth by linear measurement whether a 4, 5, 6, or 7 day period is chosen from which to compute the daily mean.

HYDROGEN-ION CONCENTRATION AS RELATED TO GROWTH

The relation of acidity and alkalinity to growth of microorganisms has been studied in detail for a large number of species. For such a study on *Fusarium lycopersici*, and on the relation of the hydrogen-ion concentration of the soil to the development of the disease, we are indebted to Scott (*49*) and Sherwood (*52*).

Scott found that maximum growth of the organism was obtained at a P_H value of 4.5 to 5.3, followed by a minimum at P_H 5.25 to 5.8, with a second maximum at P_H 5.85 to 6.85. He also found in soil experiments that a minimum of wilt occurred at a P_H of 6.4 to 7.0, with a maximum of wilt on either side. This double maximum for growth and infection has also been found by Hopkins (*28*) for *Gibberella saubinetii*. Webb (*59*) has also found double maxima in his study of spore germination as influenced by the hydrogen-ion concentration.

Sherwood (*52*) determined the range of acidity and alkalinity tolerated by his strain of *Fusarium lycopersici* in tests upon spore germination to be P_H 2.2 to 8.4, which is wider than the range of hydrogen-ion concentration found in any ordinary soil. He did not find a minimum, however, in the amount of wilt produced, with a maximum of wilt on either side in his soil experiments. He did not present data upon the dry weights of mycelium produced at different hydrogen-ion concentrations, although it is evident from his notes that there was a minimum of growth at P_H 7.6 to 8.2 in one experiment and at P_H 6.6 to 7.0 in another, with a maximum on either side. These minimum points do not agree with that found by Scott (*49*), P_H 4.5 to 5.3.

In the studies reported here, not only were the hydrogen-ion ranges of the 24 strains of Fusarium isolated from wilted tomato plants determined, but dry weights of mycelium produced at different hydrogen-ion concentrations were also obtained.

EXPERIMENTAL METHODS

CULTURE SOLUTION

A modified Richards' solution was first used in the preliminary work. This was soon discarded, however, on account of the precipitation of magnesium phosphates in the alkaline end of the series. Tarr and Noble (*56*) called attention to this precipitation of phosphates above P_H 6, and their analyses of their solutions show that the precipitation was heaviest at P_H 8.

Uschinsky's solution was next tried, but the same difficulty as with Richards' solution was found. Furthermore, the growth of the several strains tried in this solution was very poor. Apparently the carbon sources supplied—ammonium lactate, sodium asparaginate, and glycerine—were relatively unavailable for these fungi.

A solution was finally synthesized upon which the organisms made a luxuriant growth, and in which there was no magnesium. The solution consisted of the following ingredients:[7] Distilled water 1,000 c. c.; NaCl 5 gm.; H_3PO_4 24.65 c. c. normal solution; KNO_3 10 gm.; potassium acid thalate 1.020 gm.; dextrose 50 gm.

[7] In part after Tarr and Noble (*56*).

It was found that to prevent caramelization of the dextrose in the acid and alkaline solutions, it was necessary that the salt solution and the sugar solutions be made up separately, sterilized separately, and mixed when cool, under aseptic conditions. Consequently, the salt solution was made so concentrated that 40 c. c. of the new solution contained the same quantities of all the salts as 50 c. c. of the original.[8] A concentrated dextrose solution was also prepared so that 10 c. c. of the new solution contained as much dextrose as 50 c. c. of the original.[9]

PREPARATION OF CULTURE FLASKS

One hundred and twenty-five c. c. Erlenmeyer flasks were used. Forty c. c. of the salt solution was placed in each flask. The flasks were plugged and sterilized at 15 pounds pressure for 20 minutes. When cool, 10 c. c. of the dextrose solution, previously sterilized and cooled was poured in aseptically. The flasks were then incubated for 24 hours to detect contaminations.

ADJUSTING AND DETERMINING HYDROGEN-ION CONCENTRATIONS

The culture solution as prepared had a P_H value of 2.2 to 2.8. Adjustments were made by the addition of normal NaOH or normal KOH, (fig. 4). Stevens (*55*) has shown that these bases in common with NH_4OH have a low toxicity to fungous spores.
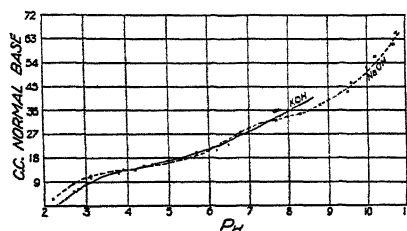
FIG. 4.—Titration curves of a modified Richards' solution with KOH and NaOH

A sample of the adjusted culture medium was sterilized with the culture flasks, and the dextrose solution was added to it in the same proportions as to the culture flasks. The hydrogen-ion concentration of this sample was determined in most cases electrometrically with the aid of the hydrogen electrode and potentiometer. In other cases the colorimetric method for the determination of hydrogen-ion concentration was used with equally satisfactory results. The color standards suggested by Medalia (*39*) were employed successfully in this connection.

INOCULATION OF CULTURE FLASKS

The flasks were inoculated by means of three loopfuls of a spore suspension containing some mycelial fragments. Wheat kernels upon which the organism had been growing for 10 to 14 days were shaken with 50 c. c. of sterile distilled water. This gave a heavy suspension of microconidia, except from the five strains in the 120-group; these gave a heavy suspension of macroconidia. The flasks after inoculation were placed in diffuse light at ordinary room temperature. Growth was allowed to proceed 101 to 10 days, after which the cultures were filtered into previously prepared, dried, and weighed Gooch crucibles. The crucibles were then dried a second time at 100 to 105° C. for 24 hours and reweighed. The difference in weights before and after filtering was assumed to be a measure of the dry weight of the mycelium

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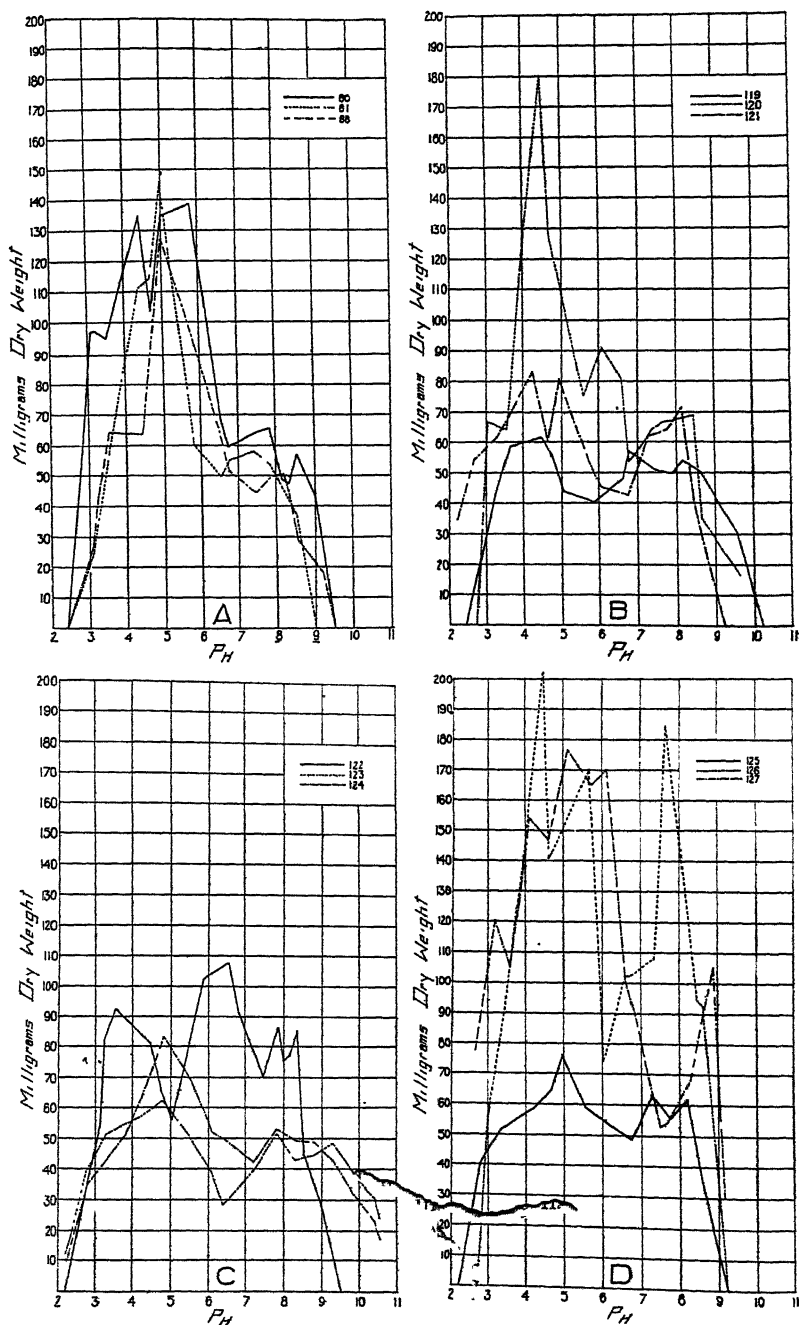
FIG. 5.—Hydrogen-ion-growth curves of *Fusarium lycopersici* strains Nos. 60, 61, 68, 119, 120, 121, 122, 123, 124, 125, 126, and 127
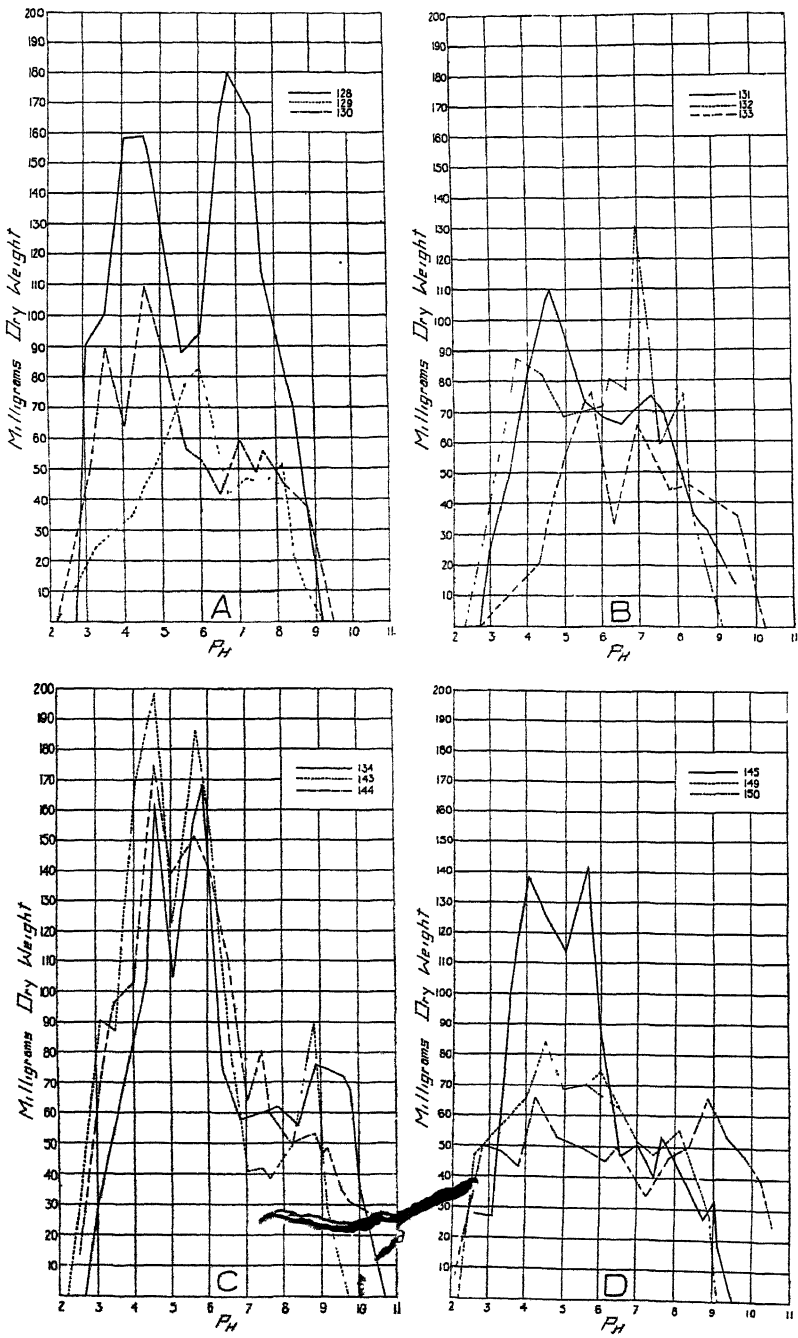
FIG. 6.—Hydrogen-ion-growth curves of *Fusarium lycopersici* strains Nos. 128, 129, 130, 131, 132, 133, 134, 143, 144, 145, 149, and 150

produced. The series were always run in duplicate, but only the average dry weight of the mycelium produced by the two cultures at any one hydrogen-ion concentration are recorded. It was from these figures that the hydrogen-ion-growth curves were plotted.

<center>EXPERIMENTAL RESULTS</center>

The experimental data obtained appear in Table 8 and in Figures 5 and 6. In Figure 7, A are presented graphically the minimum and maximum hydrogen-ion concentrations tolerated by the strains where determined, and in Figure 7, B are shown the curves obtained for one strain when the tests were repeated three times.

<center>DISCUSSION OF RESULTS</center>

In analyzing these results the expected variation in dry weight due to the methods employed should be kept in mind. Brown (8) has pointed out the disadvantages of using dry-weight determinations as measurements of fungous growth, since under apparently identical conditions wide variations will frequently occur in the amount of dry weight produced. These irregularities were found to be more common and of greater extent as the limits of alkalinity were approached, and less so toward the limits of acidity. In this work, which involved such a large number of experimental cultures, the hydrogen-ion concentration of each was not determined. The cultures were prepared as described above in groups of nine, eight of which were used

FIG. 7.—A, hydrogen-ion ranges of 24 strains of *Fusarium lycopersici;* B, hydrogen-ion-growth curves of strain 119, obtained from three repetitions at widely different times—December, 1923, March, 1924, and May, 1924

for inoculation, the ninth being reserved for the determination of the hydrogen-ion concentration. It was realized from the start that this method would involve slight errors. However, the hydrogen-ion concentrations of the groups were usually far enough apart so that no overlapping of individual cultures within two neighboring groups would result. The electrometric method of determining hydrogen-ion concentration was replaced by the colorimetric method during the latter part of these investigations. In addition to being more rapid than the electrometric method, it was accurate to within 0.1 P_H.

It should be borne in mind that these studies were conducted with several strains of the same organism, and the general appearance of the hydrogen-ion growth curves would be expected to be similar. Slight variations of the curve might be due to one of two things; either to physiological differences of the strains or to the possibility of errors arising, as just pointed out.

TABLE 8.—*Growth, expressed as dry weight in milligrams, of 24 strains of Fusarium lycopersici grown on a liquid medium for 10 days, at different hydrogen-ion concentrations*

Strain 60:																		
P_H values	2.4	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.0	9.5	10.2
Dry weight	(a)	96.4	96.0	91.2	135.0	103.8	134.8	138.2	69.4	59.8	64.2	61.6	48.9	47.6	57.3	43.0	(b)	(a)
Strain 61:																		
P_H values	2.4	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.0		
Dry weight	(a)	25.5	32.6	57.7	112.3	114.7	140.5	60.2	50.0	55.4	57.6	53.6	41.5	38.0	37.5	0		
Strain 68:																		
P_H values	2.4	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.0	9.5	
Dry weight	(a)	27.0	42.3	64.7	64.3	79.1	128.7	94.8	66.4	51.7	45.0	48.8	50.6	44.7	29.0	17.8	(a)	
Strain 119:																		
P_H values	2.4	3.1	3.2	3.5	4.4	4.6	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.0	9.5	10.2
Dry weight	(a)	(b)	42.5	58.5	61.1	128.1	44.3	40.4	47.7	57.0	51.3	50.0	54.3	65.8	47.8	41.0	30.7	(b)
Strain 120:																		
P_H values	2.7	3.0	3.5	4.0	4.4	4.6	5.5	6.0	6.6	6.7	7.3	7.6	8.4	8.5	8.7	9.2		
Dry weight	(a)	67.0	61.4	133.6	180.9	128.1	75.3	91.7	81.0	53.9	61.7	66.7	68.7	35.4	33.3	24.3		
Strain 121:																		
P_H values	2.2	2.7	3.3	4.2	4.6	5.7	6.0	6.7	7.7	8.4	9.2							
Dry weight	34.7	54.7	62.0	83.5	60.5	53.4	45.8	42.7	64.2	11.3	(a)							
Strain 122:																		
P_H values	2.2	2.8	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.5	
Dry weight	(a)	(b)	56.5	82.5	92.8	81.9	66.5	56.4	103.0	71.3	92.8	71.2	87.1	78.2	76.5	85.9	(a)	
Strain 123:																		
P_H values	2.5	2.8	3.3	3.8	4.2	4.8	5.5	6.1	6.4	7.2	7.8	8.3	8.8	9.3	10.4			
Dry weight	(b)	39.9	52.1	45.1	57.5	63.0	51.2	39.4	28.0	40.0	51.6	42.9	41.7	39.7	30.8			
Strain 124:																		
P_H values	2.5	2.8	3.3	3.8	4.2	4.8	5.5	6.1	6.4	7.2	7.8	8.3	8.8	9.3	10.4			
Dry weight	(b)	35.0	43.0	51.3	62.4	83.8	60.5	52.1	50.0	42.8	53.5	49.6	49.2	42.8	32.8	22.8		
Strain 125:																		
P_H values	2.2	2.7	3.3	4.2	4.0	4.9	5.7	6.0	6.7	7.2	7.7	8.1	8.4	8.8	9.2	10.2		
Dry weight	(b)	39.9	51.7	50.3	65.0	76.5	50.8	54.5	49.0	63.6	56.8	62.3	44.2	20.5	(*)	33.3		
Strain 126:																		
P_H values	2.7	3.0	4.0	4.0	4.4	4.6	5.5	6.0	6.5	6.6	6.7	7.3	7.6	8.4	8.3	8.7	9.2	
Dry weight	(b)	56.1	142.1	203.3	140.8	171.9	74.0	99.2	104.0	103.0	108.7	185.6	95.9	92.9	(b)			
Strain 127:																		
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.7	8.9	9.1	
Dry weight	(*)	77.6	121	105.4	154.5	147.1	177.2	165.8	170.8	102.2	78.4	54.9	68.1	106.1	55.2	30.4		
Strain 128:																		
P_H values	2.7	3.0	3.5	4.0	4.5	4.6	5.5	6.0	6.5	6.5	7.3	7.6	8.4	8.7	9.2			
Dry weight	(*)	90.4	100.5	157.7	158.1	155.6	87.6	94.2	165.0	179.9	165.1	114.4	70.2	39.8	(*)			
Strain 129:																		
P_H values	2.2	2.7	3.3	4.2	4.6	4.9	5.7	6.0	6.7	7.2	7.7	8.1	8.4	8.8	9.2			
Dry weight	(*)	14.6	24.5	35.4	45.3	51.3	78.8	82.4	41.4	47.9	46.6	50.2	20.2	11.5	(*)			
Strain 130:																		
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.1	
Dry weight	(a)	(b)	51.1	89.6	63.9	109.3	89.2	56.3	53.5	42.4	59.7	48.9	56.8	45.6	11.5	(b)	(*)	

Strain 131:															
P_H values	2.7	3.0	3.5	4.0	4.4	4.6	5.5	6.0	6.6	6.7	7.3	7.6	8.4	8.5	8.7	9.2
Dry weight	(a)	24.8	49.4	83.4	105.7	109.6	73.6	68.4	66.0	71.1	75.1	70.1	36.8	33.2	31.9	20.9

Strain 132:													
P_H values	2.8	3.7	4.4	4.9	6.0	6.2	6.6	6.9	7.5	8.1	8.3	8.4	8.6	9.1
Dry weight	29.2	87.6	82.3	68.2	71.9	80.2	76.4	130.3	58.6	75.9	40.3	32.8	8.9	(a)

Strain 133:															
P_H values	2.7	3.1	4.2	4.4	5.0	5.4	5.7	6.3	6.9	7.8	8.3	9.5	9.7	10.0	10.2
Dry weight	(a)	(b)	21.0	31.9	56.3	72.5	76.7	32.7	65.4	44.4	46.2	36.2	27.2	(b)	(a)

Strain 134:																		
P_H values	2.7	3.1	4.2	4.4	5.0	5.4	5.7	6.3	6.8	7.7	8.3	8.8	9.5	9.6	10.0	8.9	9.1	10.2
Dry weight	(a)	32.3	103.3	161.8	104.9	155.8	169.3	75.9	59.4	62.8	56.4	77.1	72.8	67.8	36.8	54.6	29.7	36.8

Strain 143:																		
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.1	9.5
Dry weight	(a)	(b)	91.9	87.6	168.9	109.4	122.8	187.3	152.3	86.2	41.3	42.2	38.6	49.8	90.0	54.6	48.4	31.1

Strain 144:																	
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.5
Dry weight	(b)	20.1	68.3	96.7	103.5	173.0	139.7	152.0	140.0	111.0	64.5	81.1	60.8	49.1	53.1	33.2	35.1

Strain 145:																	
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.5
Dry weight	(b)	27.9	27.0	97.9	138.1	125.7	113.9	141.1	92.8	47.3	50.7	40.9	53.8	45.5	27.4	33.2	(a)

Strain 149:																
P_H values	2.2	2.6	4.0	4.5	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.1	9.5
Dry weight	(b)	47.6	66.5	83.7	69.0	70.9	75.4	62.7	51.8	47.3	37.6	58.3	37.7	27.8	(b)	(a)

Strain 150:																
P_H values	2.5	2.8	3.3	3.8	4.2	4.8	5.5	6.1	6.4	7.2	7.8	8.3	8.8	9.3	9.8	10.2
Dry weight	(b)	50.1	48.6	43.1	65.6	52.7	49.3	45.5	49.7	33.6	46.6	50.5	66.6	53.1	46.4	40.3

a No growth.   *b* Slight growth.

All the strains showed a double maximum. The first maximum, while varying with the strains to some extent, appeared at P_H 4.0 to 5.5, and was followed by a minimum at P_H 5 to 7, with the second maximum at a point above P_H 7. This second maximum was found to be less regular in its position upon the P_H scale than the first. For example, the second maximum of strain 122 fell at P_H 6.6, while the second maximum of strains 134 and 143 fell at P_H 8.8. The second maximum of strain 144 as well as the minimum point was poorly defined, if present at all. Strains 122, 128, 132, and 150 are outstanding in respect to their second maximum. In these cases the second maximum is higher than the first, and in the first three cases mentioned, it fell just below neutrality. In strain 150, however, the second maximum fell at P_H 8.8, a point decidedly above neutrality.

It should be pointed out here that strain 150 in common with several others—119, 121, 122, 123, 124, 125, 129, 133, and 149—did not make the rapid and heavy growth in 10 days made by the remaining 14 strains.

Figure 7, B brings out an interesting fact observed during the course of these investigations. The maximum acidity tolerated and the first maximum growth point in the curve were found to be fairly constant, always appearing at nearly the same points on the P_H scale. However, the minimum growth point of the curve, the second maximum growth point, and also the maximum alkalinity tolerated were variable, and often varied between wide limits, especially the alkaline limit for germination and growth. The curves of Figure 7, B show these irregularities as well as the similarities of the curves obtained by three repetitions of this one strain. The irregularity of the maximum alkalinity tolerated is also expressed in graphic form in Figure 7, A.

Variations were also found in the growth ranges of the strains. For example, strain 61 has a short range (P_H 3.1 to 8.6) while others, especially strains 127 and 144, have long ranges (less than P_H 2.2 to more than P_H 10.2).

The writer has been unable to correlate the hydrogen-ion-growth curves with the temperature-growth curves or with the degrees of pathogenicity expressed by the several strains as presented in an earlier part of this paper.

SOURCES OF ENERGY FOR GROWTH

DIFFERENT SOURCES OF ENERGY

Two strains, namely, 144 and 150, were selected as representing the two arbitrary groups set up previously in this paper on a basis of virulence and macroscopic cultural characters, and were grown on Richards' solution with varying carbon sources at 2 per cent concentrations. The methods employed were similar to those previously used for measuring growth by dry-weight determinations.

The cultures were run in series of six. The figures which appear in Table 9 are the average of the dry weights obtained from the six cultures. Growth was allowed to continue for 10 days from the time of inoculation, after which the cultures were filtered into previously prepared and weighed Gooch crucibles, and dry-weight determinations were computed.

TABLE 9.—*Growth of strains 144 and 150 with different carbon sources*

| Carbon source | Dry weight | | Carbon source | Dry weight | |
	Strain 144	Strain 150		Strain 144	Strain 150
	Mgm.	*Mgm.*		*Mgm.*	*Mgm.*
Arabinose	35. 1	45. 3	Galactose	44. 5	38. 5
Dextrose, 1 per cent	45. 2	41. 2	Levulose	70. 1	64. 7
Dextrose, 2 per cent	56. 3	48. 0	Lactose	18. 7	15. 9
Maltose	61. 7	53. 1	Dextrine (white)	66. 2	58. 1
Mannose	46. 3	42. 4	Dextrine (yellow)	75. 6	85. 5
Glucose	92. 3	63. 1			

In Table 9 it is shown that strain 144, which had made more rapid growth in previous experiments, again showed greater rapidity of growth. Strain 150 produced the greater dry weight over the time allotted, on arabinose and yellow dextrine. Although the difference between the two strains is slight, yet it is constant. Lactose served as a very poor carbon source for both strains.

In this connection should be noted the very poor growth made by several of the strains on Uschinsky's solution with ammonium lactate, sodium asparaginate, and glycerine as the only available carbon sources.

UTILIZATION OF DEXTROSE AS RELATED TO HYDROGEN-ION CONCENTRATION

It was the purpose of this series of experiments to follow the use of dextrose by *Fusarium lycopersici* during the growth period when different initial hydrogen-ion concentrations were used.

EXPERIMENTAL METHODS

Growth was measured by dry-weight determinations as described previously. Hydrogen-ion concentrations were determined electrometrically. Dextrose determinations were made by the general gravimetric method as recommended by the Association of Official Agricultural Chemists (1). The culture solution used was Richards' solution.[10] This solution as prepared had an initial P_H reading of approximately 4 after sterilization. Small quantities of dextrose were destroyed by the sterilization process (15 pounds pressure for 20 minutes), so that the initial concentration was approximately 4.5 per cent in all the series.

Series 1 had an initial P_H reading of 4.04. In series 2 the KH_2PO_4 was replaced by an equal amount of K_3PO_4. The initial P_H of the solution in this series was 7.15. In series 3, K_3PO_4 was used and the resulting solution adjusted by the addition of $Ca(OH)_2$ to an initial P_H of 9.04.

The flasks were inoculated with a spore suspension of *Fusarium lycopersici*, strain 144, and kept in diffuse light at laboratory temperature. During the period of most rapid growth dry weight determinations, sugar determinations, and hydrogen-ion determinations were made every other day in triplicate. As changes between determinations became less marked determinations were made less frequently.

[10] KNO_3, 10 gms.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, 20 mgm.; dextrose, 50 gm.; distilled water, 1,000 c. c.

EXPERIMENTAL RESULTS

The data obtained on the relation between growth, hydrogen-ion concentration, and changes in hydrogen-ion concentration caused by growth, and dextrose utilization are presented in Table 10. The data are also expressed graphically in Figures 8 and 9.
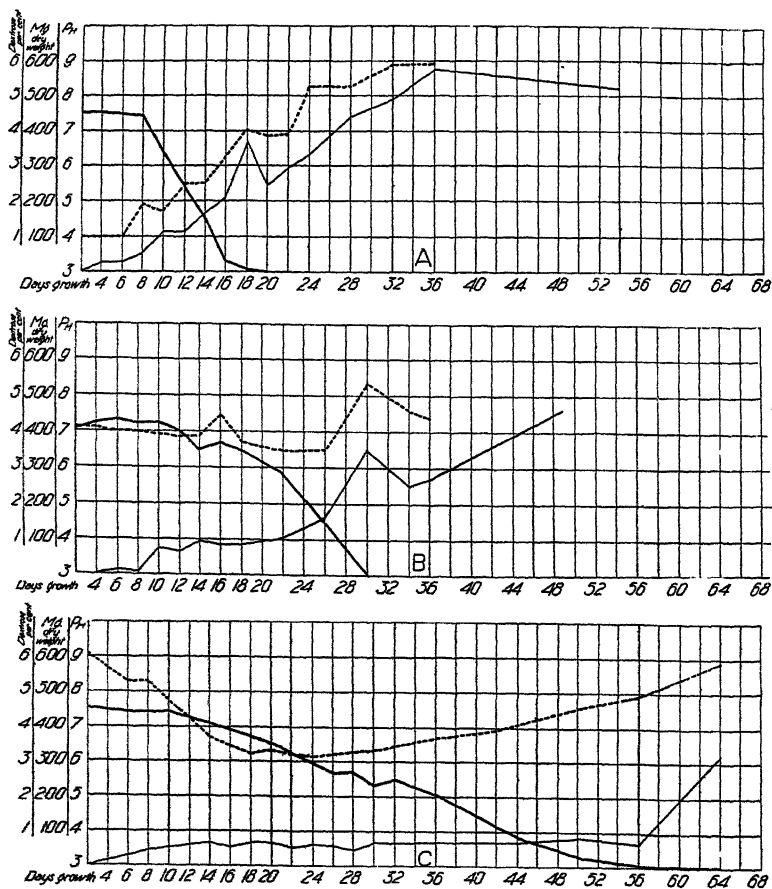
FIG. 8.—Dextrose utilization and changes in hydrogen-ion concentration as a result of the growth of *Fusarium lycopersici* on a modified Richards' solution. A, initial P_H, 4; B, initial P_H, 7; C, initial P_H, 9

DISCUSSION OF RESULTS

UTILIZATION OF DEXTROSE AS RELATED TO INITIAL HYDROGEN-ION CONCENTRATION.—With an initial P_H reading of $4 \pm$ (series 1), dextrose was used rapidly, and at the end of 20 days traces only were left which disappeared in two more days. With an initial P_H of $7 \pm$ (series 2), dextrose was used less rapidly, and measurable quantities were found at the end of 28 days, but none in 30 days. With an initial P_H of $9 \pm$, dextrose was used even less rapidly, and traces persisted after 51 days of growth.
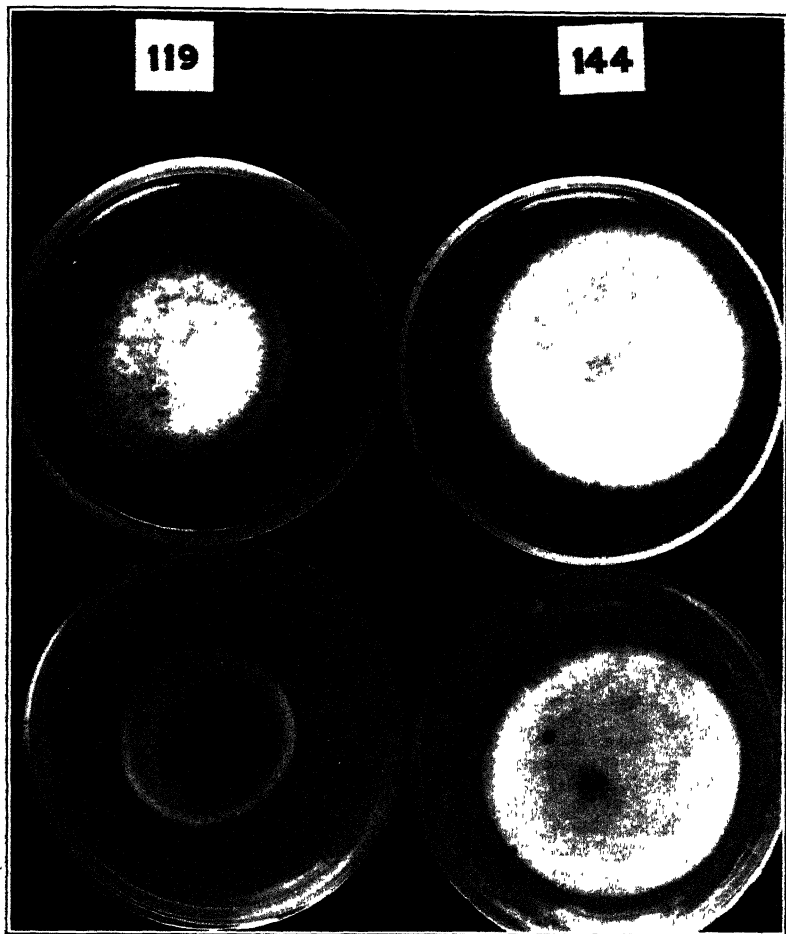
Surface (upper) and under side (lower) of culture of strain 119 (Iowa) and strain 144 (Wisconsin), grown on potato-dextrose agar at 20° C. for 10 days
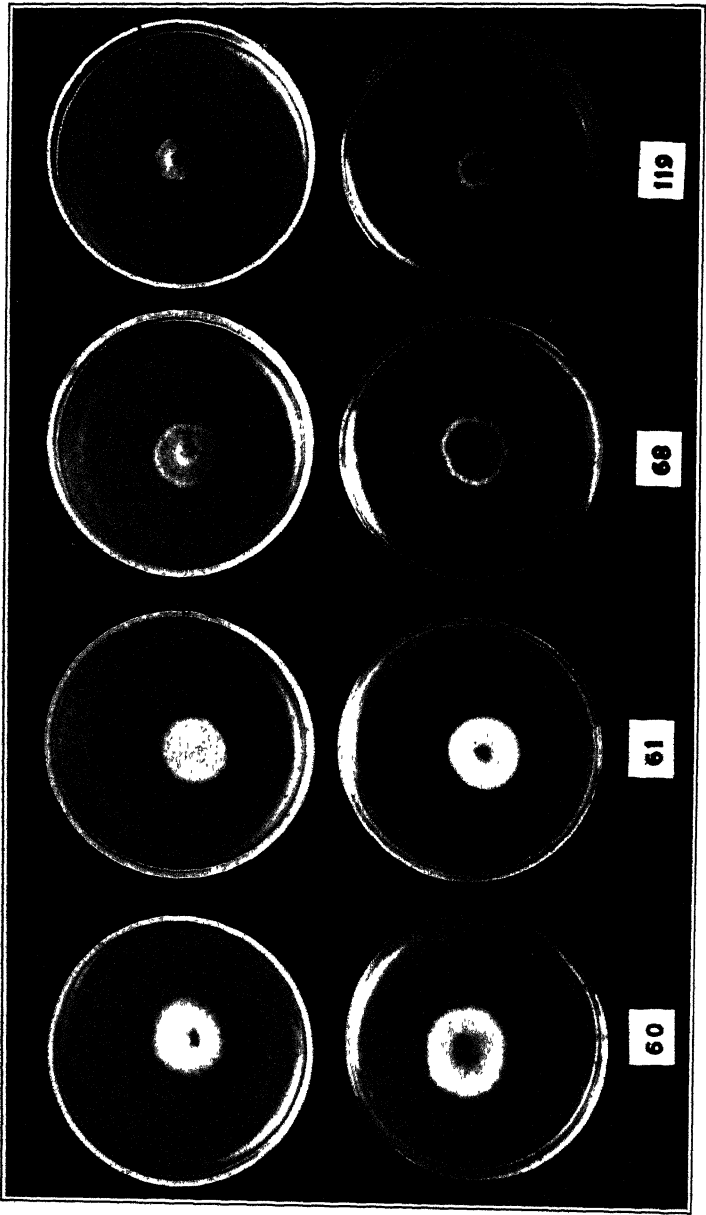
Surface (upper) and under side (lower) of cultures of strains 60, 61, 68, and 119, grown on potato-dextrose agar at 32° C. for six days
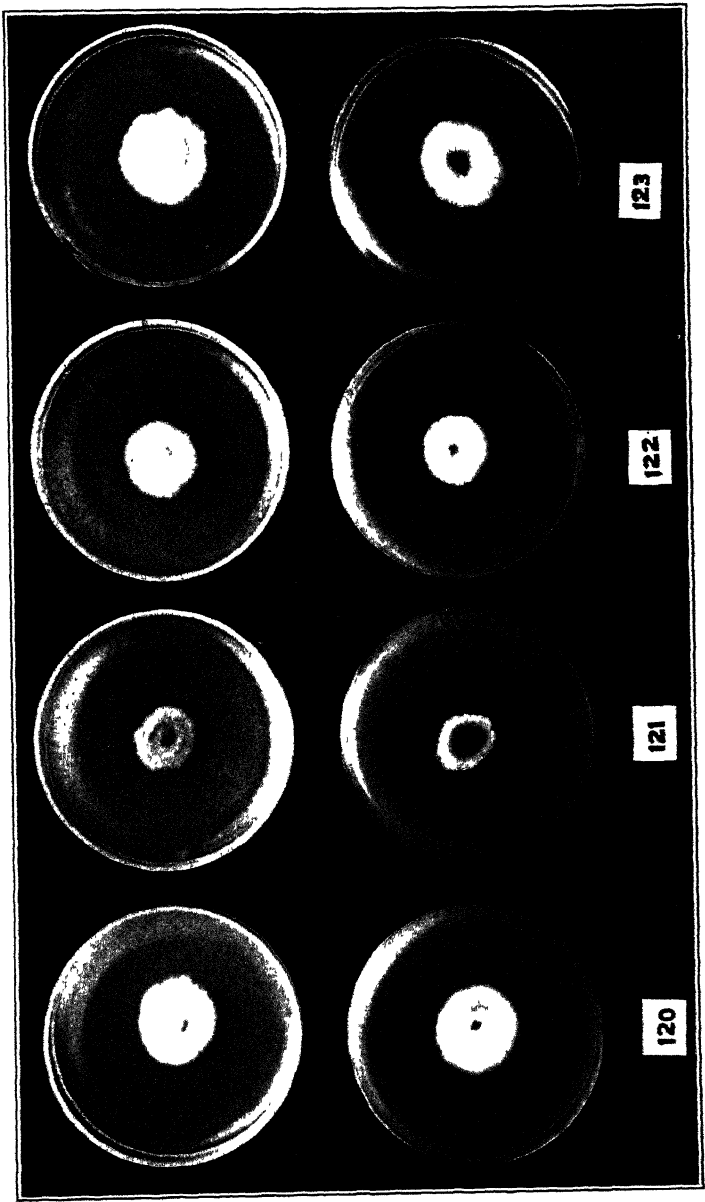
Surface (upper) and under side (lower) of cultures of strains 120, 121, 122, and 123, grown on potato-dextrose agar at 32° C, for six days
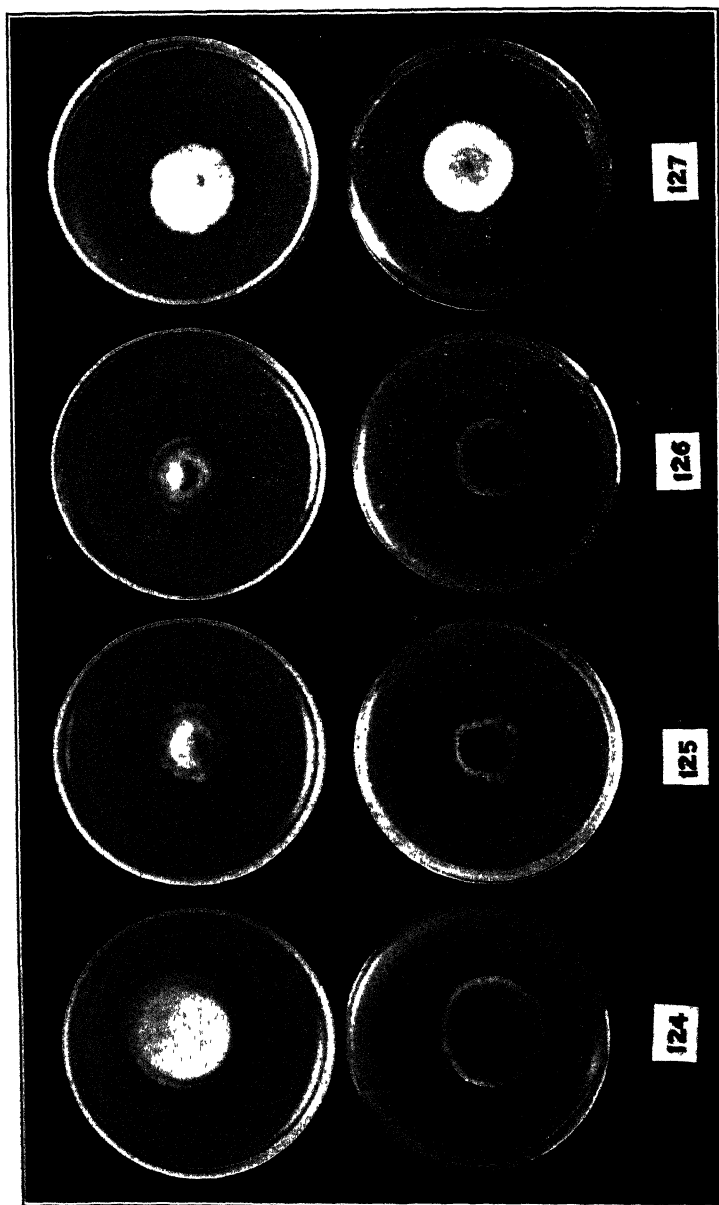
Surface (upper) and under side (lower) of cultures of strains 124, 125, 126, and 127, grown on potato-dextrose agar at 32° C. for six days
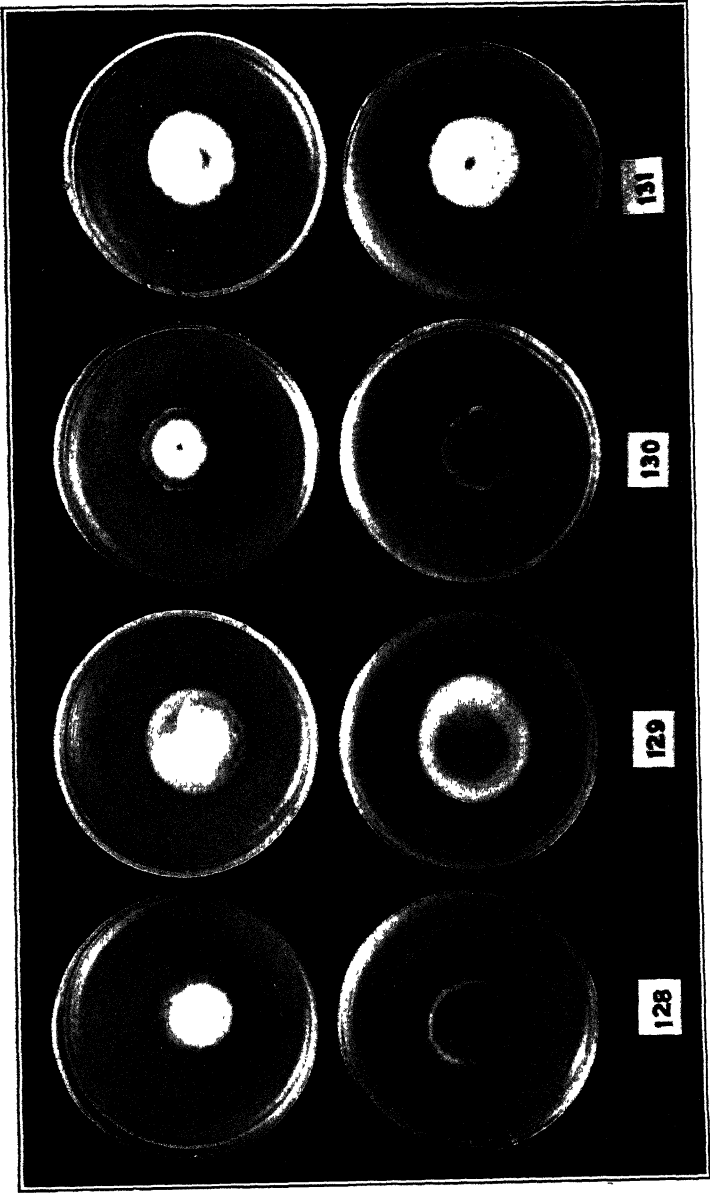
Surface (upper) and under side (lower) of cultures of strains 128, 129, 130, and 131, grown on potato-dextrose agar at 32° C. for six days
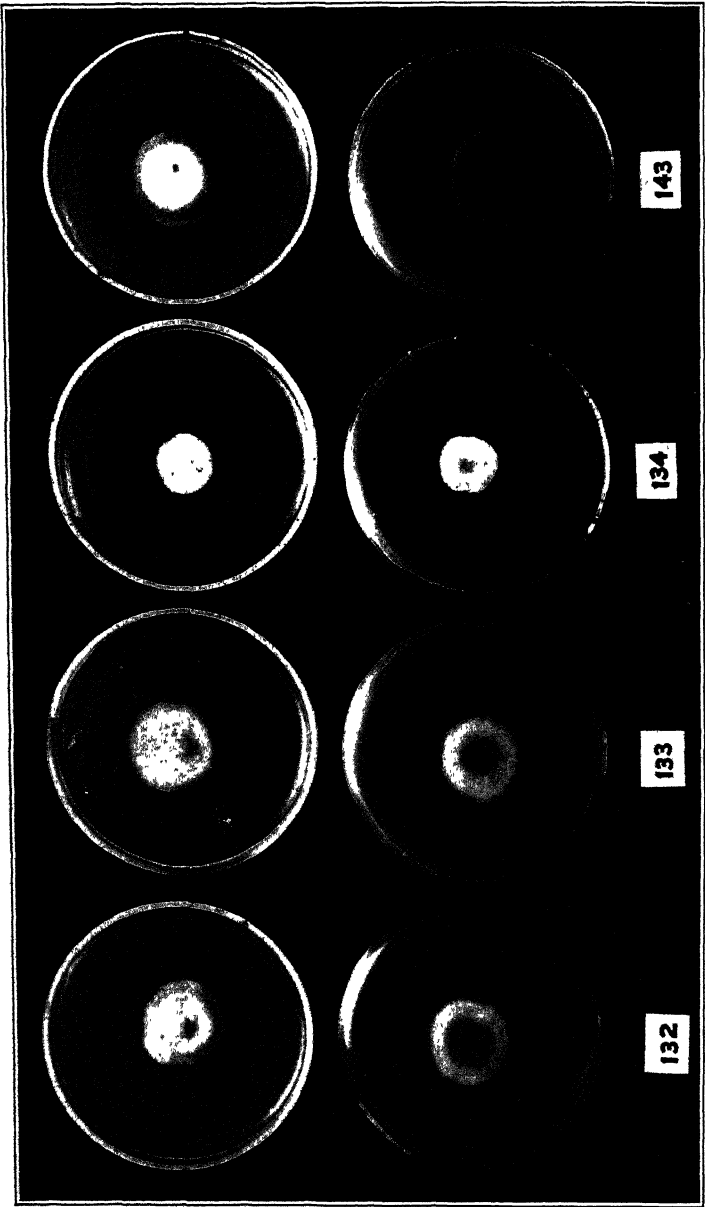
Surface (upper) and under side (lower) of cultures of strains 132, 133, 134, and 143, grown on potato-dextrose agar at 32° C. for six days
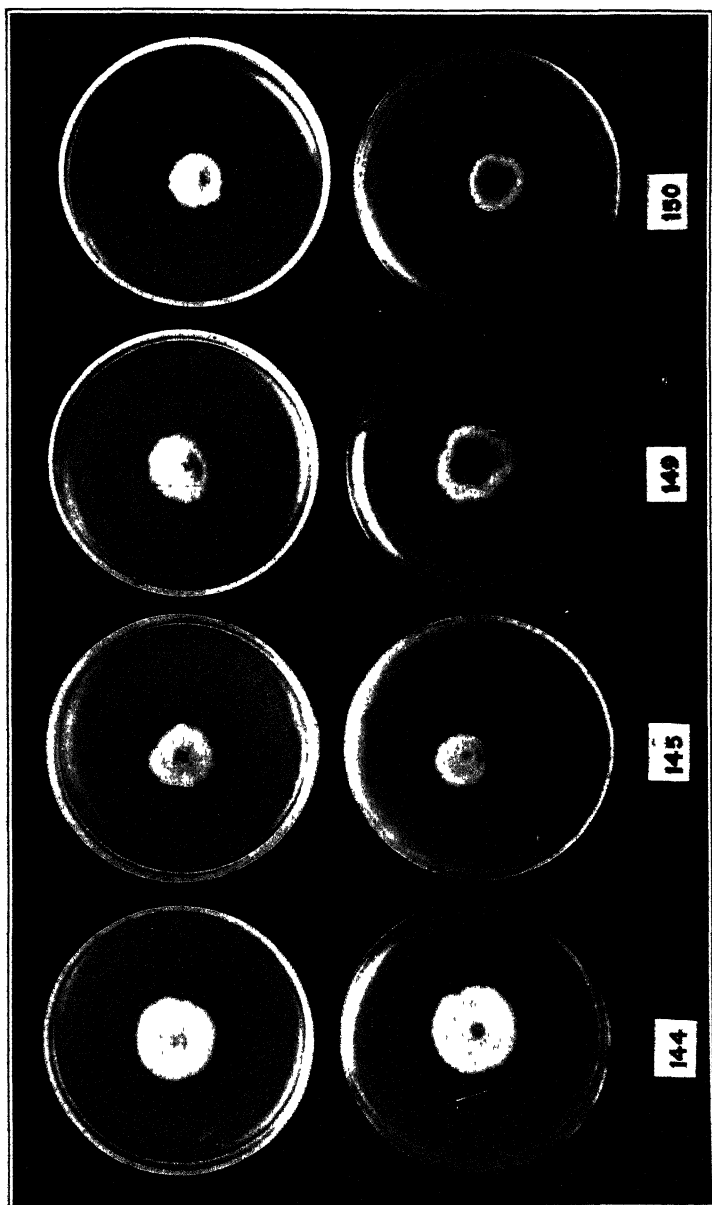
Surface (upper) and under side (lower) of cultures of strains 144, 145, 149, and 150, grown on potato-dextrose agar at 32° C. for six days

TABLE 10.—*Dextrose utilization and hydrogen-ion changes as related to growth*

Number of days	Series 1, initial P_H reading, 4			Series 2, initial P_H reading, 7			Series 3, initial P_H reading, 9		
	Subsequent P_H readings	Dry weight of fungous mat in mgm.	Per cent of dextrose	Subsequent P_H readings	Dry weight of fungous mat in mgm.	Per cent of dextrose	Subsequent P_H readings	Dry weight of fungous mat in mgm.	Per cent of dextrose
0	4.04	--------	4.50	7.15	--------	4.14	9 04	--------	4.54
4	4.04	28.1	4.54	7.174	--------	4.27	--------	--------	--------
6	4.04	25.5	4 47	7.050	14.3	4.33	8.346	37.2	4 40
8	4.942	55.5	4.48	7.050	7.4	4.22	8 361	42.9	4.40
10	4.694	110 7	3.47	6.993	79.7	4.22	7.743	54.2	4 41
12	5.438	118.1	2.43	6.847	69.7	4.05	--------	--------	--------
14	5.528	164 1	1.54	6.847	95.6	3.54	6.712	67.1	4.10
16	6.261	210.2	.31	7.444	81.2	3.68	6 452	59.2	3.88
18	7.072	363.5	Trace.	6.689	81.6	3.52	6.295	65.4	3.69
20	6.813	242.2	.02	--------	--------	--------	6.306	64.3	3.47
22	6.802	293 2	--------	6.520	102.7	2.81	6.249	57.6	3.25
24	8.233	329.3	--------	--------	--------	--------	6.159	62.2	2.91
26	--------	--------	--------	6.577	160.5	1 42	6.203	60.5	2.66
28	8.222	440.4	--------	--------	--------	--------	6.261	54.4	2 73
30	--------	--------	--------	8.301	350.3	--------	6 396	66.4	2.36
32	8.842	486.9	--------	--------	--------	--------	6.452	66.4	2.48
34	--------	--------	--------	7.602	245.6	--------	--------	--------	--------
36	8.865	572.8	--------	7.410	267.7	--------	6 666	72 2	2.09
44	--------	--------	--------	--------	462.6	--------	6.982	70.5	.88
49	--------	--------	--------	--------	--------	--------	--------	--------	--------
51	--------	--------	--------	--------	--------	--------	7.579	81.6	.34
52	--------	516.1	--------	--------	--------	--------	--------	--------	--------
56	--------	--------	--------	--------	--------	--------	7.872	67.9	Trace.
60	--------	--------	--------	--------	--------	--------	8.909	329.5	--------
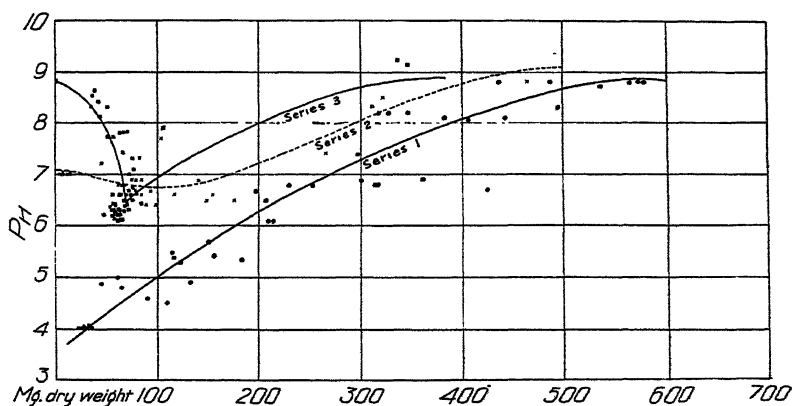
FIG. 9.—Relation of growth of *Fusarium lycopersici* to changes in the hydrogen-ion concentration of the solution

Correlated with this varied utilization of dextrose is the dry weight of the fungous mats produced. In series 1, growth was rapid from the start, after an initial lag due to the extremely small quantities of active mycelium present. Growth was less rapid in series 2, and the maximum dry weight obtained was never equal to that produced in series 1. Even more pronounced are these results in series 3. Here the dry weight produced even after long periods of growth was less than 100 mgm., the maximum being reached after about 14 days' growth. The remaining dextrose, from this time on, was apparently used only for respiration purposes, since no further increases in dry weight were recorded until after the dextrose had all been used.

The explanation of these variances seems to lie in the fact that organisms have a definite optimum hydrogen-ion concentration for growth. This optimum point was apparently reached early in the growth period of series 1. In series 2 and 3, if the optimum was ever reached, which is doutful, especially in series 3, it was reached later in the growth period, and after certain katabolic products had been formed which inhibited or retarded growth.

CHANGES IN HYDROGEN-ION CONCENTRATION CAUSED BY GROWTH.— Certain changes in the P_H readings of the solutions took place as a result of growth (fig. 8, A, B, and C). This phenomenon has been noted by many other investigators. Starting from an initial P_H of 4, the acidity of the culture solutions decreased rapidly until a P_H of 6.8 to 7 was reached. After a short time, during which all traces of dextrose disappeared, the acidity again continued to decrease until eventually a P_H of 8.8 was reached. Starting with an initial P_H of 7, only very slight changes took place in the acidity of the culture solutions until the sugar disappeared, when acidity decreased rapidly and a maximum P_H of 8.2 was reached. With an initial P_H of 9 the acidity was gradually increased as a result of growth until a minimum P_H of 6.1 to 6.2 was reached, after which the acidity gradually decreased. Between P_H 6.2 to 6.8 is the "isometabolic" point as defined by Sideris (*53*).

CONTINUED INCREASE IN DRY WEIGHT AFTER DISAPPEARANCE OF DEXTROSE.—In series 1 and 2 the fact is evident that after all traces of dextrose had disappeared from the culture solution, the fungous mat continued to increase in dry weight, without any apparent source of carbon. In series 1, for example, the dry weight at 36 days was more than twice that at 20 days, the last time dextrose could be detected in the solutions. It was thought that the dextrose might have been converted in this instance into salts of organic acids, alcohols, etc., which were subsequently used as sources of energy.

Analyses were therefore made of culture solutions 20 and 60 days old for salts of organic acids and alcohols. Both were found in larger amounts in the 20-day-old cultures than in the 60-day-old cultures, but the quantities were too small to account for the large increase in dry weight recorded for series 1. For example, salts of organic acids, as acetic, were found to be present as 0.00768 per cent in 20-day-old cultures, and 0.0024 per cent in 60-day-old cultures; the alcohols, 0.391 per cent by volume in 20-day-old cultures, and 0.0018 per cent in 60-day-old cultures.

In a duplicate series with Richards' solution, ethyl alcohol being used as the only source of carbon, 77.3 mgm. dry weight of *Fusarium lycopersici* were produced on a 0.5 per cent solution, the dry weight decreasing as the concentration of the alcohol either increased or decreased from this point. The continued production of dry matter by this organism after the sugar had disappeared could be explained in part, at least, by the conversion of some of the dextrose to ethyl alcohol and its subsequent use. Salts of organic acids probably play a less important part in this respect.

The thallus of 20-day-old cultures was very granular. The cells of the hyphae were filled with globules which gave the microchemical tests for glycogen only faintly. These globules of substance disappeared in older hyphae, and in cultures 60 days old could be found

only in the youngest hyphal tips.   This suggested to the writer that the sugar as used was not all employed for the building up of protoplasm, but that a considerable part was stored in the cells for future use.

TOXIC EXCRETORY PRODUCTS PRODUCED BY FUSARIUM LYCOPERSICI, AND THEIR RELATION TO TOMATO WILT

INTRODUCTION

It has been the opinion of plant pathologists that fungi and bacteria causing the wilt diseases of various hosts produce wilting by mechanically plugging or otherwise obstructing the lumina of the fibrovascular bundles, thereby interfering with the free passage of water from the -roots to the leaves (*12, 22, 36, 41, 42, 54*).   This conception of wilting is still found in literature published on the subject of wilts (*9, 16, 23, 45, 46*), and has much in its favor, especially since the appearance of the paper by Melhus et al. (*40*) upon this subject.   Other explanations, however, have been advanced, chief among which are: (1) Decay of roots, causing decreased absorption, cutting the water supply down to a point where the host can no longer function normally; (2) the nutrition theory, which is based upon the supposition that the organisms growing within the lumen of the vessels deplete the liquids passing through of mineral elements necessary for normal growth of the host; (3) the toxic theory, based upon the supposition that the organisms concerned, as a result of growth, produce toxic excretory substances which cause the death of the host protoplasm.

It was noted that plants that had been infected with a wilt-producing pathogene often showed browned fibrovascular bundles from which the organism could not be isolated (*26*).   It was further noted that the hyphae very rarely, if ever, were packed closely enough in the lumina to seriously hinder the passage of water (*5, 57*). These and other facts were instrumental in the formulation of the theory that toxic excretory products were produced by the pathogene which were injurious to the cells of the host.

REVIEW OF LITERATURE

Haskell (*26*) has shown that decomposition products of the dead cells of the potato are able to discolor the fibrovascular bundles and are toxic to the cells with which they come into contact.   He also showed that fibrovascular browning could be produced by the injection of certain toxic substances into the lumina of the bundles. Overton (*43*) and Dixon (*14*) concluded that the products of plant cells were toxic to the plants themselves, and that wilting might be due to the introduction of poisonous or plasmolyzing substances into the transpiration stream.

Tisdale (*57*), in his studies on flax wilt, has pointed out that partial destruction of the root system by the pathogene will interfere with the normal intake of water from the soil and cause a wilting under semidrought conditions.   As early as 1892, Atkinson (*2*) pointed out that the fungus growing in the lumina of the fibrovascular bundles of cotton, and causing a wilt of that host, used a part of the food material which normally went to the host.   This nutritional loss was thought to be a factor in the production of a wilted condition.   The work

herein reported is concerned primarily with a study of the toxic excretory products produced by *Fusarium lycopersici.*

Brandes (5) was one of the first to demonstrate this toxic principle. He grew *Fusarium cubense* on Richards' and Uschinsky's solutions for two weeks. The cultures were then filtered aseptically. Cut stems of buckwheat plants, bean plants, and banana leaves were immersed in the filtrate, in sterile solution, and in distilled water. Wilting was found to be very rapid in those plants immersed in the filtrate, while the controls in sterile solution and distilled water remained turgid. He concluded from these experiments that wilting was not due to the plugging of the vessels, but was probably the result of toxic excretions by the fungus.

Fahmy (18), working on the toxic excretory products of *Fusarium solani,* concluded that the substance which was toxic and produced the wilting was thermostable and nonvolatile. Barnum (4) has shown that *Penicillium expansum,* classed by him as a strict saprophyte, is also capable of producing excretory substances which are toxic to plants. This substance or these substances are concluded to be thermostable and nonvolatile.

Picado (44) has made a study of the exo and endotoxins of *Fusarium solani, Verticillium albo-atrum, V. duboys,* and a saprophytic Penicillium. He concluded from his studies that wilting, browning of the vessels, and dissolution of tissue were not always due to the same cause. This conclusion is based upon the fact that wilting of plants was often more pronounced in heated extracts than in those not heated. This could not be the case if wilting were due entirely to substances of the nature of enzymes.

From previous studies on the toxic principles involved, it seemed to the author that probably two distinct toxic principles might be present. The earlier work on this subject indicates that the toxic substance is enzymic in nature. The later work of Fahmy (18) and Barnum (4), however, would lead one to believe otherwise, since they state that the toxic principle is thermostable, admittedly not a character of an enzyme.

The experiments reported below show that there are two such toxic principles involved. One is enzymic and therefore thermolabile; the other is crystalloidal and thermostable.

EXPERIMENTAL METHODS

In these experiments strain 134 was used. The organism was pure lined by the usual methods and was kept in culture on potato-dextrose hard agar with frequent transfers from the time it was isolated until it was used. This strain was found to be strongly pathogenic to susceptible varieties of tomatoes in the greenhouse.

The culture solution chosen was a modification of Richards' solution. Brandes (5) suggested that when sucrose was used as a source of carbon for fungi, it was broken down to glucose and fructose before being used by the organism. One molecule of sucrose thus gives rise to one molecule each of glucose and fructose. This would double the osmotic pressure of the solution if complete reduction took place and if none of the glucose or fructose were used in the metabolism of the organism. In any case, the osmotic pressure of the solution after growth of the organism upon it, would be increased. This

A, cut stems of John Baer tomato plants immersed in sterile Richards' solution with the indicated dilutions; plant on right, control in distilled water; photographed after 24 hours

B, cut stems of John Baer tomato plants immersed in the filtrate from a 4-weeks-old culture of *Fusarium lycopersici* on Richards' solution, with the indicated dilutions; plant on right, control in distilled water, photographed after 24 hours

C, John Baer plants grown in Knop's solution for eight weeks, Knop's solution then replaced by sterile Richards' solution with the indicated dilutions; plant on right, control in distilled water; photographed after 24 hours

D, same as C, except that Knop's solution was replaced by the filtrate from a 4-weeks-old culture of *Fusarium lycopersici* on Richards' solution with the indicated dilutions; plant on right, control in distilled water.
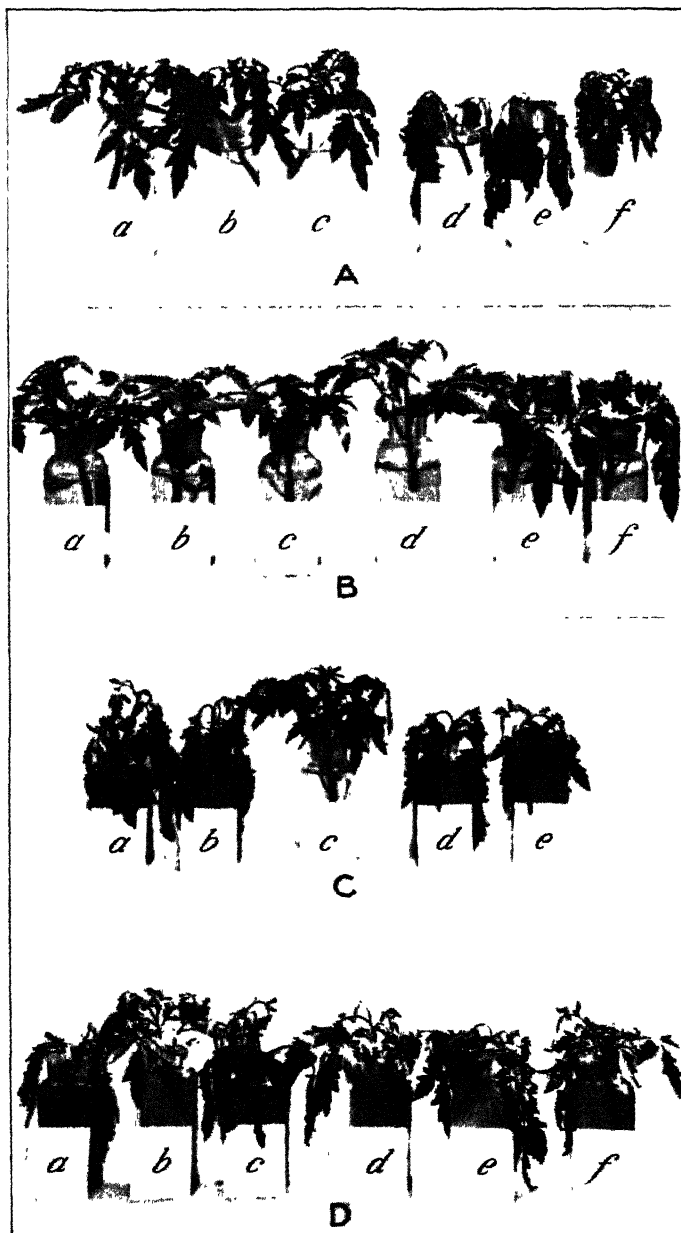
A, a, b, c, cut tomato stems of John Baer plants immersed in distilled water; d, e, f, John Baer plants immersed in distilled water solutions of the intracellular enzymes extracted from a mycelial mat of *Fusarium lycopersici*; photographed after five hours.

B. Treatment of plants similar to that in A except that enzyme solution used was boiled for 10 minutes; photographed after 24 hours

C, a, b, cut tomato stems of John Baer plants immersed in dialyzate; d, e, in diffusate from a 2-weeks-old culture of *Fusarium lycopersici* on Richards' solution; c, control in sterile Richards' solution diluted to 50 per cent of its original concentration; photographed after 30 hours.

D. Repetition of experiment shown in C; a, b, c, in dialyzate; d, e, f, in diffusate; photographed after 50 hours.

was divided into three 200 c. c. lots and placed in wide-mouthed bottles. Young vigorous tomato plants were cut under water and immersed immediately in the solutions. Three similar plants were immersed in distilled water and used as controls.

At the end of five hours the plants immersed in the enzyme solutions were badly wilted, while those in distilled water were turgid (pl. 9, A). The enzyme solution was removed and boiled for 10 minutes. A flocculent grayish-white precipitate resulted. The boiled enzyme solution was again divided into three 200 c. c. lots and placed in wide-mouthed bottles as before. Young tomato plants were immersed as before, both in the solutions and in the distilled water. All the plants remained turgid and showed no signs of wilting at the end of 24 hours (pl. 9, B). The toxic properties of the substances extracted from the mycelial mat are evident, and have properties similar to those possessed by enzymes. Exactly similar results were obtained when the enzymes contained in the filtrate were precipitated with 95 per cent alcohol and used in water solutions.

Several plants of the Black Eyebrow soybean and Early Buff cowpea were immersed in both the intra- and extra-cellular enzyme solutions used above. When examined at the end of two hours, the soybeans were very badly wilted, while the cowpeas were only slightly wilted.

EXPERIMENT 4.—TEST OF BOILED AND UNBOILED FILTRATE

From the preceding experiments it appeared that the toxic properties of the culture solutions upon which *Fusarium lycopersici* had been grown were due to enzymes secreted by the fungus. It seemed, therefore, that this toxic property if entirely enzymic in nature could be completely destroyed by boiling the filtrate. Consequently, the filtrate from a 4-weeks-old culture was divided into two portions. Each portion was divided into three parts. One part of each was kept at its original strength, a second part diluted to half its original strength with distilled water, and the third part diluted to one quarter of its original strength with distilled water.

The three parts of one portion were now boiled for 15 minutes. The solutions were placed in 250 c. c. wide-mouthed bottles, and stems of young, vigorous tomato plants, cut under water, were immersed in them. As a control, similar plants were immersed in sterile Richards' solution. In five hours all the plants in both the boiled and unboiled filtrate were badly wilted. The control plants remained turgid for 24 hours. Boiling the filtrate did not deactivate the toxic principle, a fact which led the writer to suspect that a thermostable substance toxic to tomato plants was also present in the filtrate. The substance could not be enzymic in nature, since enzymes are destroyed by boiling. The possibility suggested itself that the thermostable substance was crystalloidal in character. The following experiment was conducted with this idea in mind.

EXPERIMENT 5.—TOXICITY OF DIALYZED FILTRATE

A 2-weeks-old culture of *Fusarium lycopersici* was filtered in the usual manner through a Büchner funnel. Three cultures produced 750 c. c. of filtrate. This was placed in a dialyzer made of two thicknesses of parchment paper. The filtrate was covered with

a thin film of toluene to prevent contamination. This dialyzer was suspended in 750 c. c. of distilled water. At the end of 60 hours, the diffusate was amber colored as was the dialyzate. Because of the higher osmotic pressure of the filtrate, water passed into the parchment bag, so that at the end of 60 hours only 600 c. c. of the diffusate was recoverable, while the dialyzate had increased in volume to 900 c. c. These were siphoned off into 250 c. c. wide-mouthed bottles, and cut stems of young vigorous tomato plants of a suscep- tible variety were immersed in them at once. Controls consisted of similar plants immersed in sterile Richards' solution diluted to 50 per cent of its original strength. This dilution was made in order that approximately the same osmotic pressure relationships would exist over the whole series. Wilting was slight in all plants within five hours, with the exception of the controls. The condition of the plants in this series at the end of 30 hours is shown in Plate 9, C. Plants in both the diffusate and dialyzate were equally wilted at the termination of this time. The solutions were subjected to 15 minutes boiling, after which freshly cut stems of tomato plants were immersed as before. Wilting occurred as above, both in the diffusate and dialyzate, although not so rapidly as before boiling. This experiment was repeated with similar results (pl. 9, D).

EXPERIMENT 6.—OSMOTIC PRESSURES IN RELATION TO TOXICITY OF SOLUTIONS USED IN PREVIOUS EXPERIMENTS

It was deemed advisable at this stage of the studies to repeat the work already done in its entirety, before further work was attempted. Consequently, freshly cut, 6-weeks-old tomato plants of the John Baer variety were immersed in the following solutions: Distilled water, tap water, distilled water plus the extracellular enzymes from the filtrate of a 3-weeks-old culture of *Fusarium lycopersici*, distilled water plus the intracellular enzymes obtained from the mycelial mats of two 3-weeks-old cultures of *F. lycopersici*, pure filtrate from a 3-weeks-old culture; filtrate diluted 50 per cent with distilled water, filtrate diluted 75 per cent with distilled water, diffusate from two 3-weeks-old cultures, dialyzate from the same source, pure sterile Richards' solution, sterile Richards' solution diluted 50 per cent with distilled water, and sterile Richards' solution diluted 75 per cent with distilled water.

The osmotic pressures of all the solutions except the tap and distilled waters were determined cryoscopically with the aid of a Beckmann freezing apparatus. Notes were taken at intervals upon the behavior and appearance of the plants. The data obtained appear in tabular form as Table 11. It was unfortunate for the experiment that the day the solutions were ready to use was cloudy. Bright days induce rapid transpiration and, therefore, a rapid rise of the toxic principles into the plant parts through the transpiration stream and result in bringing about a more rapid wilting. However, the results were satisfactory and confirmed those previously obtained.

TABLE 11.—*Behavior of 6-weeks-old John Baer tomato plants when cut and immersed in various solutions*

Solution	Osmotic pressure in atmospheres	General appearance of the plants after—		
		2 hours	4 hours	24 hours
Distilled water	0 0	Turgid	Turgid	Turgid.
Tap water		do	do	Do.
Distilled water plus extracellular enzymes from filtrate of F. lycopersici culture	1.44	Wilted	Badly wilted	Very badly wilted.
Distilled water plus intracellular enzymes from mycelial mats.	.48	Badly wilted	Dead	Dead.
Pure filtrate	11.68	Turgid	Wilted	Do.
Filtrate diluted 50 per cent with distilled water.	5 90	do	Slightly wilted	Very badly wilted
Filtrate diluted 75 per cent with distilled water.	3.13	do	Turgid	Do.
Diffusate	6.38	do	do	Badly wilted.
Dialyzate	5 05	do	do	Do.
Pure sterile Richards' solution	12 16	do	do	Turgid.
Sterile Richards' solution diluted 50 per cent with distilled water.	6 02	do	do	Do.
Sterile Richards' solution diluted 75 per cent with distilled water.	2.41	do	do	Do.

It should be explained that after 24 hours in the extracellular enzyme solution, the bundles were browned for 6 inches into the stem. In the case of the intracellular enzyme solutions, the bundles of the plants immersed were also browned and the leaves were dry and brittle. Attempts were made to isolate an organism from these browned fibrovascular bundles, but the results were negative. The stems of plants immersed in the dialyzate and all dilutions of the filtrate were watersoaked and slimy in appearance as contrasted with the shriveled but firm stems of the plants immersed in the diffusate. After 24 hours all these plants were very badly wilted.

These experiments were repeated in part with a new set of solutions with very similar results. The plants immersed in distilled water and in sterile Richards' solution remained turgid for 48 hours, while those immersed in the enzyme solutions were badly wilted at the end of three hours and were dead and dry at the end of 30 hours. The plants immersed in the dialyzate and diffusate from a 3-weeks-old culture were equally wilted at the end of 30 hours, but the plants in the dialyzate wilted sooner than those in the diffusate. Those plants immersed in the filtrate were badly wilted at the end of 30 hours, those in the pure filtrate being dead and dry at the end of that time.

EXPERIMENT 7.—TOXICITY OF FILTRATES TO RESISTANT AND SUSCEPTIBLE VARIETIES

This experiment was designed to test the effects of the filtrate from a 3-weeks-old culture of *Fusarium lycopersici* grown on Uschinsky's solution on susceptible and resistant varieties of tomatoes. Three wilt-producing organisms were used: *F. lycopersici* from St. John, Kans., strain 134; *F. lycopersici*, from Manhattan, Kans., strain 133; and *F. oxysporum* Schlecht, obtained from G. B. Ramsey. At the end of three weeks' growth (which was very poor) of these organisms on Uschinsky's solution, the cultures were filtered in the usual manner and the filtrate placed in pint fruit jars. As a check upon

the behavior of the plants immersed in the filtrates, plants were immersed in sterile Uschinsky's solution. Young tomato plants, 8 to 10 inches in height, of the varieties Comet and Kanora were used. The former variety is susceptible, while the latter is highly resistant. The young plants were grown in pots and were cut under water and transferred at once to the filtrates, three plants being placed in each jar. The results obtained are shown in Table 12.

At the end of one hour wilting was evident in all the plants except the controls. The Comet plants were more wilted than the Kanora, although all are classed as slightly wilted in the table. At the end of two hours, the Comets in the filtrate from the culture of *F. oxysporum* were badly wilted, while the Kanora plants were still only slightly wilted. At the end of six hours the difference in wilting between plants of the two varieties was even more evident. The resistant Kanora plants still showed slight wilting; the susceptible Comets were badly wilted in the filtrates of *F. lycopersici,* and very badly wilted in the filtrate from *F. oxysporum.*

TABLE 12.—*Behavior of Comet and Kanora tomato plants immersed in filtrates from cultures of wilt-producing Fusaria*

Solution	Variety	Appearance of plants after—		
		1 hour	2 hours	6 hours
Sterile Uschinsky's solution	Kanora	Turgid	Turgid	Turgid.
	Comet	do	do	Do.
Filtrate No. 133	Kanora	Slightly wilted	Slightly wilted	Slightly wilted.
	Comet	do	do	Badly wilted.
Filtrate No. 134	Kanora	do	do	Slightly wilted.
	Comet	do	do	Badly wilted.
Filtrate *F. oxysporum*	Kanora	do	do	Slightly wilted.
	Comet	do	Badly wilted	Very badly wilted.

EXPERIMENT 8.—DISTILLATION OF FILTRATES

To determine the volatility of the nonenzymic toxic substance or substances, distillation of the filtrate was carried out. One liter of filtrate from a 6-weeks-old culture of the organism on Richards' solution was fractionally distilled. Eight 100 c. c. fractions were collected, and cut stems of tomato plants of the John Baer variety were immersed in them. The first fraction proved highly toxic, wilting of the plants immersed in it occurring within two hours. The second fraction was much less toxic and the remaining six fractions were nontoxic. The residue was highly toxic. Evidently both volatile and nonvolatile substances are present which are toxic to cut tomato plants.

EXPERIMENT 9.—TESTS FOR TOXICITY ON CUT TOMATO STEMS OF SUBSTANCES ISOLATED FROM SOLUTIONS UPON WHICH FUSARIUM LYCOPERSICI HAD BEEN GROWN

It has previously been pointed out in this paper (p. 222) that solutions upon which *Fusarium lycopersici* were grown, contained varying amounts of alcohol and salts of organic acids. In order to determine if these substances were toxic to cut tomato stems, and were possibly concerned in the wilting of tomatoes infected with this organism,

solutions of organic acids and ethyl alcohol were prepared in distilled water.   Cut tomato stems were immersed in these solutions and their behavior noted.

Ethyl alcohol was not toxic to cut tomato stems in concentrations up to 4 per cent.   Higher concentrations were not used.   The plants immersed in alcohol solutions remained turgid, and after four days in the solutions showed no ill effects.   During this time large quantities of the solutions had been transpired.

The organic acids used were acetic, proprionic, normal butyric, and normal valeric, of the fatty-acid series; and lactic, oxalic, tartaric, and benzoic.   All of these acids proved extremely toxic to cut tomato plants.   All except lactic and tartaric acids produced rapid wilting and death of the plants at as low concentrations as 0.06 of 1 per cent. At this concentration lactic and tartaric acids produced slight wilting only at the end of four hours, while the other acids had caused death of the seedlings at this concentration in a much shorter time.   Lactic and tartaric acids were highly toxic at 0.12 of 1 per cent concentration.   The cells of the stems of plants immersed in oxalic acid were disintegrated.   None of the other acids had this effect.   Benzoic acid bleached the stems of all color.

It is clear that these acids in very low concentrations are highly toxic to tomato plants, and may be a factor in the wilting of plants caused by vascular parasites.

<center>DISCUSSION</center>

From the foregoing experiments, it is evident that culture solutions upon which *Fusarium lycopersici* has grown are toxic to tomato plants.   Some light has been thrown upon the nature of this toxic principle by a study of some of its properties.   It has been found that there are at least two substances or groups of substances with certain opposite characters capable of causing wilting in tomato plants.   Whether or not the same substances are elaborated within the host as are elaborated by the fungus when grown on artificial culture media, has not been investigated.   The action of the organism upon the cells of the host may result in the production of toxic substances entirely distinct from those produced by the organism when grown unassociated with the host.   That products of dead plant cells may be toxic to the plant itself has been shown by Haskell (*26*).   However, the experiments on the wilting of susceptible and resistant varieties of tomatoes when immersed in filtrates of cultures of *F. lycopersici* would indicate that plants of the variety Kanora, resistant to wilt under field conditions, are also more resistant to the toxic substances elaborated by the fungus in pure culture than are plants of the susceptible variety Comet.   Resistance may possibly be due, therefore, to substances within the host protoplasm which offset or counteract the injurious effects of the toxic excretory products of the pathogene.

These toxic excretory products fall into two categories; one of which has been determined to be colloidal in nature, nondialyzable, and thermolabile.   In all probability this is of the nature of an enzyme and is both extra- and intra-cellular in character.   From the experiments on dialysis of the filtrate, it was found that the diffusate was also capable of bringing about rapid wilting and death of cut

tomato plants when immersed in it. Boiling either the dialyzate or diffusate did not deactivate the toxicity of the solutions. That there is present a thermostable toxic substance, crystalloidal in nature, is also proved by the experiment with boiled and unboiled filtrate. That this crystalloidal substance is not of the nature of a coenzyme is evident from the dialyzing experiments. When separated from the nondialyzable portion of the enzyme, coenzymes lose their enzymic properties. Therefore, the diffusate of these experiments should be nontoxic if the dialyzable substance or substances were coenzymic in nature. Since this was not the case, we are led to assume that these two toxic principles are distinct from each other. We would expect, therefore, that the plants that had been grown in water culture and whose roots were then bathed in the filtrate from an old culture of *Fusarium lycopersici* would wilt as a result of the diffusion into the roots of the crystalloidal toxic substance. Barnum (4) obtained wilting of cabbage plants whose roots he claimed to be uninjured when immersed in filtrates from cultures of *Penicillium expansum*. It seems to the writer, however, doubtful whether a young plant growing in sand could be removed without breaking some of the roots. Mix [12] obtained wilting when plants grown in water culture were transferred to filtrates of a number of organisms.

In the light of the experiments reported herein the writer is unable to explain why the plants grown in Knop's solution in experiment 2 and then transferred to a filtrate from an old culture of *F. lycopersici* did not wilt.

No attempt has been made to identify the enzymes extracted. Some of the substances present in the filtrate in true solution have already been mentioned. Lathrop (*32*) has shown that *Fusarium cubense* EFS. produces a volatile aldehyde when grown on Uschinsky's solution. He suggests that this aldehyde may be toxic. Volatile substances are also concerned in the case of tomato wilt, since the first two 100 c. c. fractions of distillation proved toxic to cut tomato plants. This is in agreement with the results of Fahmy (*18*) on *F. solani* Ap. et Wr., who also demonstrated the presence of ammonia and of an oxalate in the filtrate from his cultures. These substances were found in small quantities only, and were not concentrated enough to cause the wilting which he observed.

Of the substances found in true solution in liquid cultures upon which *Fusarium lycopersici* has been grown, alcohol was proved to be nontoxic to tomato plants. Organic acids, however, salts of which are present, were proved to be extremely toxic in low concentration. The extreme toxicity of some of these organic acids in concentrations of 0.06 of 1 per cent over short periods of time would indicate their toxicity in still lower concentrations over longer periods of time. Whether or not these acids are formed in the normal relationships between the host and parasite is unknown, and has not been investigated.

The toxic substances demonstrated in these experiments are evidently nonspecific in character. Those produced by the growth of *Fusarium lycopersici* on Richards' solution were found to cause wilting of cowpeas, soy beans, and cabbages as well as tomatoes. The filtrate from a culture of *F. oxysporum* was found to be more

[12] A. J. Mix, University of Kansas, in conversation with the author.

toxic to cut tomato plants than was the filtrate from cultures of two
strains of *F. lycopersici*. It seems probable that many fungi during
their growth secrete growth products toxic to plants if present in
the transpiration stream. As yet the rôle of these toxic excretory
products in the production of a diseased condition is little understood,
and the nature of these products even less so. It is the belief of the
writer that the actual damage caused by the presence of the fungus
in the lumina of the vascular bundles has been overestimated, and
the injury done by toxic substances produced by the organism and
diffusing into the transpiration stream underestimated. In other
words, the systemic invasion of a host by a wilt-producing organism
would be of less importance if the toxic substances produced were
either absent or counteracted.

It seems likely that the resistance of certain tomato varieties to
wilt may be due in part to the presence of substances within the host
protoplasm capable of uniting with or adsorbing the enzymes or
toxins elaborated by the parasite, and thus annulling their destruc-
tive action. That we have been unable to develop varieties of
tomatoes immune to wilt is evidence in support of this theory.
Resistant varieties, theoretically, possess substances within the
protoplasm which counteract the destructive influence of the en-
zymes or toxins secreted by the parasite and upon which the organism
depends for its parasitic habit. However, these substances may have
no effect upon the crystalloidal, diffusable toxic substance which has
also been shown to cause wilt of tomato plants. It is for such wilt-
producing substances that Picado (44) would search for antidotes.
Past experience on the injection of substances into hosts to produce
a condition of immunity to plant diseases has been on the whole
unsatisfactory. Before an antidote could be intelligently prepared
for these crystalloidal toxic substances, their chemical nature should
be fully understood. Future investigations should be conducted
along these lines, so that the nature of resistance and susceptibility
to tomato wilt may be more fully understood.

GENERAL CONSIDERATIONS

The results obtained by the study of 24 isolations of Fusaria from
wilted tomato plants have emphasized the fact that isolations of the
same organism from widely different regions or localities may be
expected to differ in virulence and in certain other physiological
characters. Only in a general way have the different degrees of
pathogenicity been correlated with temperature relations and growth
rates, and these facts could not be correlated with very evident differ-
ences expressed by the strains toward the hydrogen-ion concentra-
tions of the substrate upon which they were being grown. The
statement of McClintock (35) and others, however, that strains of
tomatoes resistant to wilt in one locality are not always adapted to
other localities, has some additional experimental evidence in its
support. That this might be due to differences in the pathogene
rather than to differences in the environment was suspected by
Edgerton (15) and Clayton (11), who found that strains of the patho-
gene from different localities reacted differently toward tomato
varieties. That such strains vary in at least two physiological
characters has been shown by these studies.

By a study of the temperature relations and growth rates, the 24 strains were divisible into two groups; one group with a wider temperature range and more rapid growth rates, consisting of five members, one each from New York, Wisconsin, and Texas, and two from Louisiana, and the other group of 19 members with a more limited temperature range and slower growth rates. The wider temperature ranges and more rapid growth rates expressed by the first of these groups was correlated with a higher degree of virulence than that expressed by the members of the other group. It was further correlated with certain cultural characters as described in the text.

By a study of the hydrogen-ion-growth curves, differences in the behavior of the several strains were noted, but it was impossible to group them or to correlate these differences with the differences found in virulence, growth rates, temperature relations, or cultural characters, as far as these were studied. Nine strains were noted, however, which evidently failed to use dextrose as efficiently as a carbon source as the remaining 15 strains, judging from comparative dry weights produced over a 10-day period on a liquid medium.

Scott (*49*) has expressed the opinion that soils might be adjusted in their reaction so that they would show a hydrogen-ion concentration equal to that at which the minimum of growth of the organism and consequently the minimum of infection occurs. While he was unable to accomplish this in one field experiment owing to the fact that his soil treatments did not reach the depth to which the roots of the plants penetrated, Hopkins (*28*) was able to accomplish it with soils in the greenhouse inoculated with *Gibberella saubinetii* and on which wheat was grown. Provided the hydrogen-ion growth curve of the organism in question has been determined, this is theoretically, if not practically, a method of partial control of the disease under investigation. However, without the hydrogen-ion growth curve of the organism involved, this method may be as much of a danger as a possible benefit. For example, the minimum point in the growth curve of strain 128 in these experiments (fig. 6, A) occurs at P_H 5.6 to 6.0. The maximum of strain 129 occurs at the same range. To adjust the soil at the minimum point for one strain would be to adjust it at the maximum point for the other.

In this connection, the results of the pathogenicity studies reported in this paper may be questioned. The reaction of the soil used was not determined, and it is quite likely that the soil reaction permitted a maximum growth of some of the strains, and consequently a maximum of infection and disease, while it allowed a minimum of growth with a minimum of infection and disease for other strains. The comparative virulence of the strains might have been more accurately determined by conducting such tests at the optimum points for each of the several strains. Had this been done the several strains might have appeared equally virulent. However, it should be mentioned that the five most virulent strains, namely, 120, 122, 127, 131, and 144, showed in their hydrogen-ion growth curves different maximum points.

The fact that certain substances toxic to the hosts are produced by pathogenes and nonpathogenes when grown in pure culture, is nothing new. Whether these pathogenes produce the same or even similar toxic substance when in relation to their hosts is questionable

and as yet unproved. Further study upon this subject might profitably be directed to a study of the chemical relations between the host and parasite.

SUMMARY

The temperature relations, growth rates, and hydrogen-ion relations of 24 strains of Fusaria isolated from wilted tomato plants from various parts of the United States were studied.

The strains showed different degrees of virulence toward seedling tomato plants in greenhouse tests.

The 5 strains, Nos. 120, 122, 127, 131, and 144, were found to be especially virulent in their attack upon tomato seedlings in these greenhouse tests, and markedly more destructive than the remaining 19 strain. They were classed as a group, known as the 120 group. The other strains were classed as a second group known as the 60 group.

Strains in the 120 group exhibited slightly wider temperature ranges and more rapid growth rates than the strains in the 60 group.

Strains in the 120 group differed from the strains in the 60 group in color production on potato-dextrose agar and in the production of macroconidia on wheat kernels, but were similar to the strains in the 60 group when grown on steamed rice, steamed potato plugs, steamed tomato stems, and potato agar.

The strains used in these experiments were found to be of the nonstaling type of fungi when grown on potato-dextrose agar, but were of the staling type when grown on liquid synthetic media.

It was found in determining the mean daily increment increases of the mycelial disks that a 4, 5, 6, or 7 day growth period could be used from which to obtain the mean.

The hydrogen-ion relations of the 24 strains were determined. In general the hydrogen-ion growth curves were similar to each other.

Two maximum growth points in the curves were evident. The first maximum point was rather consistent in its position upon the scale, but the minimum growth point, the second maximum, and the limit of alkalinity tolerated were found to be more or less variable.

The limits of acidity and alkalinity tolerated were determined approximately as P_H 2.2 to 3.1 and P_H 8.6 to 10.4, varying with the strain. The first maximum appeared at a range of 4.5 to 5.5, the minimum in the curve usually appeared at 6 to 6.5, and the second maximum usually above P_H 7, but in three cases appeared just below neutrality.

Four strains showed their second maximum point the larger of the two. In one of these cases the second maximum occurred at P_H 8.8, while the other three showed their second maximum at or just below neutrality.

Nine strains used dextrose as a source of carbon less efficiently than the remaining 15. All strains grew very poorly on Uschinsky's solution, in which the carbon source is supplied as small quantities of sodium asparaginate, ammonium lactate, and glycerine.

When *Fusarium lycopersici* is grown on Richards' solution, with a P_H of 4, the acidity is decreased until a P_H of 8.8 is reached, after which no more growth takes place and no further decreases of acidity are noted. When it is grown on Richards' solution with an initial

acidity of P_H 7, slight increases in acidity occur up to the time the sugar disappears, after which acidity is decreased rapidly to P_H 8+. When it is grown on Richards' solution with an initial acidity of P_H 9, increases in acidity take place with little growth until a minimum P_H of 6.1 to 6.2 is reached. This acidity remains constant until after the sugar disappears, when acidity is decreased rapidly, reaching a final P_H of 8+. The higher the initial P_H within the limits noted, the slower is growth and sugar utilization.

When *Fusarium lycopersici* is grown on a modified Richards' solution it produces substances which are highly toxic to cut tomato plants, causing them to wilt and die in a short time.

These toxic substances have been separated by physicochemical means into two classes: The first colloidal, thermolabile, and of the nature of an enzyme; the second crystalloidal, dialyzable, thermostable, volatile, and nonvolatile.

Salts of organic acids were present in old culture solutions and organic acids were proved to be highly toxic to tomato plants.

Plants of a resistant strain of tomato were found to be less subject to injury from these toxic materials than plants of a susceptible variety.

These toxic substances produced by *Fusarium lycopersici* also caused wilting of cowpea, soybean, and cabbage. The toxic substances produced by *F. oxysporum* caused wilting of tomato plants.

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A COMPARISON OF SOME PHYSICAL AND CHEMICAL TESTS FOR DETERMINING THE QUALITY OF GLUTEN IN WHEAT AND FLOUR[1]

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INTRODUCTION

When flour is made into dough and fermentation is allowed to take place the gluten stretches and forms eventually, when the dough is baked, the supporting structure of the loaf of bread. The character of the resulting loaf, or, in other words, the baking value of the flour, is determined in part by the peculiar properties of the principal flour proteins, glutenin and gliadin, which are capable, in the presence of water, of forming a tough, coherent, and elastic mass.

Inasmuch as these gluten proteins constitute a fairly definite and uniform portion of the total protein in wheat flour, the Kjeldahl method (*2, p. 5–6*)[3] or its modification has proved a fairly satisfactory and dependable index of the quantity of gluten present.

In spite of the immense amount of research devoted to the subject, not as much as should be is known about the gluten quality of flour, although notable contributions have been made to the subject recently.

Outstanding among the tests used by different laboratories in obtaining indices of gluten quality are those for kernel texture, the washed-gluten, the water-absorbing power of the flour, the percentage of crude protein in the wheat or flour, and of late the viscometric procedure of Sharp and Gortner (*17*). Many short cuts and modifications of the last-named test have been tried, with a view to applying it as a routine method for estimating gluten quality in wheat or wheat flours.

With the exception of a recent paper by Blish and Sandstedt (*4*), very few data have been found that compare methods for rating gluten quality with the observed baking data on the same flour. The subject matter of this paper, therefore, concerns itself with a study of the results obtained by several methods now in use for determining gluten quality and compares these data with the baking data on the same samples of flour. The baking methods used are described in United States Department of Agriculture Bulletin No. 1187 (*21*). All bakings were made on straight grade flours.

For ease in comparing the value of each physical or chemical test as an indicator of gluten quality, the data obtained from a study of each method have been worked up on a statistical basis, by the Pearson method (*15, p. 373*). Such correlations are of practical value, as they place the cereal chemist in a better position to judge

the value of certain determinations in their application to the milling and baking industry.

Samples of wheat and flour representing the two crop years 1923 and 1924 were studied. The samples of the 1923 wheat crop were commercial samples that had been graded at the various Federal grain supervision offices located in the hard red spring and hard red winter wheat areas. The great majority of the 1924 wheat crop samples were grown by the Office of Cereal Investigations, Bureau of Plant Industry. They represent pure varieties of well-known hard red spring and hard red winter wheats.

In making these studies each wheat and each flour sample had all of these tests applied to it in so far as time and material permitted. Other accessory data were accumulated as the investigation progressed and are introduced from time to time to support the discussion.

Tests were made comparing the texture (or percentage of dark, hard, and vitreous kernels in the wheat) with the volume of the loaf baked from flour milled from the same lot of wheat; the protein [4] content of the wheat and of the flour milled from the wheat and the associated baking tests; the percentage of wet and dry gluten in the various flours and the volume of the loaf baked from these flours; the water-absorbing power of the flours and the volume of the loaf baked from these flours; and the viscosity as measured by the viscometric methods of Sharp and Gortner (*17*), with and without modifications; and the volume of the baked loaf of bread.

TEXTURE OF KERNEL AS AN INDEX OF BAKING QUALITY

It has long been known that there is a relationship between dark and vitreous kernels of wheat and their protein content. As has been reported earlier by the authors (*5*) and recently by Shollenberger and Coleman (*20*), this relationship is particularly noticeable if the dark, spotted, or starchy kernels are removed from the same sample of wheat and analyzed.

Frank (*8*) and Mangels and Sanderson (*13*) have reported figures comparing the protein content of different lots of wheat and the percentage of dark and vitreous kernels in these lots, and their conclusions are that high protein content is not always associated with a high percentage of dark, hard, and vitreous kernels.

Correlation studies made in the grain research laboratory, Bureau of Agricultural Economics, comparing the protein content and the percentage of dark, hard, and vitreous kernels in 500 samples of hard red spring and hard red winter wheat grown in the crop years 1923 and 1924, gave the coefficients shown in Table 1.

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[4] The usual procedure of multiplying the percentage of nitrogen by the factor 5.7 and expressing the result as protein has been adopted. It should be pointed out that approximately 25 per cent of such protein is nongluten in nature, and the protein figures listed really represent crude protein.

TABLE 1.—*Correlation between protein content of wheat and the percentage of dark, hard, and vitreous kernels of hard red spring and hard red winter wheats, crop of 1923 and 1924*

Number of samples	Percentage of protein			Percentage of dark, hard, and vitreous kernels			Coefficient of correlation	Probable error	Class of wheat	Crop year
	Average	Maximum	Minimum	Average	Maximum	Minimum				
128	12. 65	15. 20	9. 20	81. 0	100. 0	25. 2	+0. 6414	+0. 0351	Hard red spring_____	1923
113	13. 70	18. 89	9. 50	89. 5	99. 9	18. 0	+. 3981	+. 0536	_____do_____	1924
183	12. 00	15. 90	8. 20	67. 5	99. 9	6. 0	+. 6004	+. 0319	Hard red winter_____	1923
76	12. 90	18. 00	8. 40	78. 4	100. 0	18. 2	+. 5593	+. 0532	_____do_____	1924

The coefficient of correlation obtained from 183 samples of hard red winter wheat grown in 1923 was $+0.6004 \pm 0.0319$, whereas that obtained from 76 samples of the 1924 crop was $+0.5593 \pm 0.0532$. From a study of 128 samples of the 1923 hard red spring wheat crop a coefficient of $+0.6414 \pm 0.0351$ was obtained, as compared with $+0.3981 \pm 0.0536$, obtained from 113 samples of hard red spring wheat of the 1924 crop.

In each of the above cases the relationship was such that it was apparent that an estimation of the percentage of dark, hard, and vitreous kernels was only a general index of the protein content of the wheat. A low percentage of dark kernels did usually indicate a low protein content, and sometimes a high percentage of dark and hard kernels was associated with high protein content, but as an exact measure of the protein content of wheat this determination is not reliable.

But inasmuch as it is now recognized that quality of gluten, as well as quantity, is a factor influencing baking strength and that high quality is usually associated with the hard and vitreous kernels, it was decided to compare the texture of the wheat samples with the baking data obtained with the same samples. Tables 2 and 3 give the distribution of the data, as well as the correlation coefficients obtained from this comparison. Complete data are given for the bakings made of 183 samples of the hard red winter 1923 wheat crop and 113 samples of the hard red spring 1924 wheat crop.

TABLE 2.—*Correlation between kernel texture and loaf volume of hard red winter wheat, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of dark, hard, and vitreous kernels										
	5–14. 9	15–24. 9	25–34. 9	35–44. 9	45–54. 9	55–64. 9	65–74. 9	75–84. 9	85–94. 9	95 and over	Total
1,700 to 1,799_____	_____	_____	_____	_____	_____	1	_____	_____	_____	_____	1
1,800 to 1,899_____	_____	_____	_____	_____	_____	1	_____	1	1	3	6
1,900 to 1,999_____	2	_____	_____	2	2	3	4	4	1	8	26
2,000 to 2,099_____	_____	_____	3	7	6	7	12	5	1	6	47
2,100 to 2,199_____	_____	1	3	7	3	8	17	4	3	4	50
2,200 to 2,299_____	1	_____	3	4	6	5	5	3	6	2	35
2,300 to 2,399_____	_____	_____	_____	_____	_____	_____	1	2	2	2	7
2,400 to 2,499_____	_____	_____	_____	_____	_____	_____	_____	1	7	0	8
2,500 to 2,599_____	_____	_____	_____	_____	_____	_____	_____	_____	1	1	2
2,600 to 2,699_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	1	1
Total_____	3	1	9	20	17	25	39	20	22	27	183

$r = +0.0990 \pm 0.0494$.

TABLE 3.—*Correlation between kernel texture of wheat and loaf volume of hard red spring wheat, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of dark, hard, and vitreous kernels										
	0–9.9	10–19.9	20–29.9	30–39.9	40–49.9	50–59.9	60–69.9	70–79.9	80–89.9	90–100.0	Total
1,900 to 1,999								1	3	5	9
2,000 to 2,099		1			1			1	6	3	12
2,100 to 2,199						1	1	3	7	19	31
2,200 to 2,299								3	3	12	18
2,300 to 2,399					1	1	- -----	4		12	18
2,400 to 2,499								1	-- ----	5	6
2,500 to 2,599									1	5	6
2,600 to 2,699									1	4	5
2,700 to 2,799										4	4
2,800 to 2,899							1		1		2
2,900 to 2,999										1	1
3,000 to 3,099										1	1
Total		1			2	2	2	13	22	71	113

r=+0.1517±0 0620.

The distribution tables listing the data for the 128 samples of the 1923 hard red spring wheat crop and for the 76 samples of the 1924 hard red winter wheat crop are not shown, as they do not add to the information. Suffice it to say that the correlation coefficients were +0.2612 ±0.0556 for the 128 samples of hard red spring wheat and +0.0287 ±0.0773 for the 76 samples of hard red winter wheat.

The coefficients obtained from the 183 samples of the 1923 hard red winter wheat crop and from the 113 samples of the 1924 hard red spring wheat crop—namely +0.0990 ±0.0494 and +0.1517 ±0.0620, respectively—are of only minor significance. It is evident, therefore, that texture of kernel is of questionable value as an index of baking strength.

In making the above conparisons only the loaf volume of the bread has been considered as an index of gluten quality. It is well known, however, that loaf volume can be materially increased at the expense of the texture of the crumb. An attempt was made to offset this contingency by taking 100 points as a perfect crumb-texture score and by making, for every 1 per cent drop in the quality of crumb texture a 1 per cent deduction from the observed volume of the baked loaf. That is to say, the loaf volume in cubic centimeters was multiplied by the crumb-texture score of the loaf, and the resulting figure was compared with the kernel texture of the wheat in the same manner in which the original loaf volume was compared with the precentage of virteous kernels. When this method of assigning baking values was used, the coefficients reported above for the 1923 and 1924 hard red winter wheat crop changed from +0.0990 ±0.0494 and +0.0287 ±0.0773 to +0.1027 ±0.0493 and +0.0328 ±0.0773 respectively.

For the samples of the 1923 and 1924 hard red spring wheat crops the coefficients changed from +0.2612 ±0.0556 and +0.1517 ±0.0620 to +0.2937 ±0.0545 and +0.1249 ±0.0625. This method of valuation did not materially change the coefficients; thus, no change in the earlier deductions can be made.

PROTEIN CONTENT OF WHEAT AND LOAF VOLUME OF BREAD

During the last few years the protein content of wheat has been assuming increased importance in determining the market price of wheat. Transactions are known where as much as 30 to 40 cents a bushel premium were paid for wheat of the same numerical grade but of higher protein content.

Such investigations as those of Zinn (*26*), Bailey (*3*), Thomas (*24*), Stockham (*23*), Sherwood (*18, 19*), and Mangels (*12*), wherein the protein content of the wheat has been compared with the baking data, have given evidence which tends to substantiate the practice and value of judging baking strength by a determination of the protein content of the wheat. It is agreed that there are individual cases in which the relationship between the percentage of protein in the wheat and the associated baking test does not hold because of the influence of other factors that affect baking strength; yet, when purchases are made on a large scale and the wheat is blended, the individual differences seem to disappear, and a close relationship is shown to exist.

In Tables 4 and 5 are given the distribution data and the correlation coefficients obtained by comparing the protein content of 183 samples of hard red winter wheat grown in 1923 and of 116 samples of hard red spring wheat grown in 1924 with the associated loaf volumes.

TABLE 4.—*Correlation between protein content of wheat and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in wheat									
	8–8.99	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	Total
1,700 to 1,799	1									1
1,800 to 1,899			2	1	3					6
1,900 to 1,999	1	5	2	6	4	7	1			26
2,000 to 2,099	4	1	5	23	10	2	2			47
2,100 to 2,199			1	5	23	12	7		2	50
2,200 to 2,299				5	13	9	8			35
2,300 to 2,399						2	4	1		7
2,400 to 2,499					1	4	1	1	1	8
2,500 to 2,599							1	1		2
2,600 to 2,699								1		1
Total	6	7	19	66	41	32	7	4	1	183

$r = +0.4281 \pm 0.0407$

TABLE 5.—*Correlation between protein of wheat and loaf volume of hard red spring wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in wheat										
	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	17–17.99	18–9 18.9	Total
1,900 to 1,999	1	2	2		2	1	1		1		10
2,000 to 2,099		4	3	3	1	2	1				14
2,100 to 2,199	1	4	5	3	4	7	4		2		30
2,200 to 2,299			1	7	4		5	1	1		19
2,300 to 2,399			1	3	3	4	3	3	1		18
2,400 to 2,499				1	1	2	2				6
2,500 to 2,599			1		2	1	1	1			6
2,600 to 2,699					2	1	1	1			5
2,700 to 2,799					1	1		1	1		4
2,800 to 2,899						1	1				2
2,900 to 2,999										1	1
3,000 to 3,099							1				1
Total	2	10	13	17	20	20	20	7	6	1	116

$r = +0.4184 \pm 0.0517$

A study of Tables 4 and 5 will show that a positive, somewhat significant relationship exists between the protein content of the wheat and the volume of loaf made from it. With the samples of the 1923 hard red winter wheat crop the coefficient between the protein content and loaf volume was +0.4281 ±0.0407, and with those of the 1924 hard red spring crop it was +0.4184 ±0.0517.

The coefficient of correlation between the protein content of the 1924 hard red winter wheat crop and the associated loaf volume was −0.0370 ±0.0773.

An examination of Table 6 shows that there was a decided relation between the loaf volume of the bread and the protein content of the wheat up to and including the 12 per cent protein area. When the 13 per cent area was reached, however, there was a decided break in the quality of the gluten, as 20 of the 34 samples of wheat having 13 per cent of protein and over produced loaves of bread having a volume of 1,999 cubic centimeters or less. From a statistical angle, therefore, these samples counterbalanced the very high relationship which would have been found with the samples of wheat that had a protein content of 8 to 12 per cent.

A coefficient of +0.4610 ±0.0407 was obtained when the baking data were compared with the protein content of the 128 samples of spring wheat flour from the crop of 1924.

When the loaf-volume figures were corrected for variations in the texture of the crumb, the coefficients were slightly smaller. In the case of the 183 samples of the 1923 hard red winter wheat crop the coefficient changed from +0.4281 ±0.0407 to +0.3932 ±0.0422. For the 116 samples of the 1924 hard red spring wheat crop the coefficient changed from +0.4184 ±0.0517 to +0.3694 ±0.0546. The coefficient −0.0370 ±0.0773 for the 76 samples of hard red winter wheat of the crop of 1924 changed to −0.0353 ±0.0773, and the coefficient +0.4610 ±0.0470, obtained from the 128 samples of hard red spring wheat, crop of 1923, changed to +0.4068 ±0.0498, when texture of crumb was considered.

TABLE 6.—*Correlation between protein content of wheat and loaf volume of hard red winter wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in wheat											
	8–8.99	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	17–17.99	18–18.99	Total
1,600 to 1,699	2											2
1,700 to 1,799	1							1				2
1,800 to 1,899	3	1						4	1	4		13
1,900 to 1,999		5		1	1	1	1	1	5	2		17
2,000 to 2,099		5	1		2	1	1	2			1	13
2,100 to 2,199		1	2	2	4	4			2			15
2,200 to 2,299			1	5	1				1			8
2,300 to 2,399				1	1							2
2,400 to 2,499								1				1
2,500 to 2,599					2	1						3
Total	6	12	4	9	11	7	2	9	9	6	1	76

$r = -0.0370 \pm 0.0773$.

PROTEIN CONTENT OF FLOUR AND LOAF VOLUME OF BREAD

Inasmuch as only 75 to 80 per cent of the crude protein content of wheat is really gluten protein, studies were next made comparing the protein content of straight grade flours with the associated loaf volumes just discussed. These data are given in full for the samples of the 1923 hard red winter and 1924 hard red spring wheat crops. The distribution data and coefficients of correlation are shown in Tables 7 and 8.

Although it would seem reasonable to expect a higher coefficient of correlation by plotting protein content of flour against loaf volume, slightly lower coefficients were obtained in each case, namely, +0.4249 ±0.0409 for the 183 flour samples of the 1923 crop of hard red winter wheat and +0.4005 ±0.0526 for the 116 samples of the 1924 hard red spring wheat. The coefficient obtained for 76 samples of the hard red winter wheat crop of 1924 was +0.1153 ±0.0763, whereas a coefficient of +0.4641 ±0.0468 was obtained for the 128 samples of the hard red spring wheat crop of 1923.

TABLE 7.—*Correlation between protein content of flour and loaf volume of hard red winter wheat, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in flour									
	7-7.99	8-8.99	9-9.99	10-10.99	11-11.99	12-12.99	13-13.99	14-14.99	05-15.99	Total
1,700 to 1,799	1									1
1,800 to 1,899			2	1	3					6
1,900 to 1,999	2	2	5	6	3	7	1			26
2,000 to 2,099	4	3	9	19	7	3	2			47
2,100 to 2,199		2	9	19	11	5	3	1		50
2,200 to 2,299			7	13	9	5	1			35
2,300 to 2,399					1	5	1			7
2,400 to 2,499				1		1	4	1	1	8
2,500 to 2,599							1	1		2
2,600 to 2,699							1			1
Total	7	7	32	59	34	26	14	3	1	183

$r = +0.4249 \pm 0.0400.$

TABLE 8.—*Correlation between protein content of flour and loaf volume of hard red spring wheat, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in flour										
	8-8.99	9-9.99	10-10.99	11-11.99	12-12.99	13-13.99	14-14.99	15-15.99	16-16.99	17-17.99	Total
1,900 to 1,999	1	1	1	2	1	2					8
2,000 to 2,099		5	2	2	2	1	3				15
2,100 to 2,199			7	7	1	5	6	4	1		31
2,200 to 2,299			1	9	1	2	3	2	1		19
2,300 to 2,399			1	4	2	3	4	4		1	19
2,400 to 2,499				1		2	3				6
2,500 to 2,599		1			1	1	1	1	1		6
2,600 to 2,699						1	2	1			4
2,700 to 2,799						1	1	2			4
2,800 to 2,899				1		1					2
2,900 to 2,999									1		1
3,000 to 3,099							1				1
Total	1	7	12	26	8	19	24	14	4	1	116

$r = +0.4005 \pm 0.0526.$

When the differences in texture of the crumb were taken into consideration, lower coefficients were obtained in every case. The coefficient for the 183 samples of the hard red winter wheat crop of 1923 changed from $+0.4249 \pm 0.0409$ to $+0.3816 \pm 0.0426$; that for the 116 samples of 1924 hard red spring wheat, from $+0.4005 \pm 0.0526$ to $+0.3278 \pm 0.0559$; that for the 76 samples of 1924 hard red winter wheat, from $+0.1153 \pm 0.0763$ to $+0.0464 \pm 0.0772$; and that for the 128 samples of the 1923 crop of hard red spring wheat, from $+0.4641 \pm 0.0468$ to $+0.3856 \pm 0.0508$.

It appears from the above data, therefore, that under the conditions under which these samples were baked, nothing is gained by making a protein determination on the flour rather than on the wheat. Samples of very low protein content were generally associated with low loaf volume. On the other hand, wheats containing over 13 per cent of protein appeared to contain a weaker quality of protein or gluten than those containing 11 or 12 per cent.

WET AND DRY GLUTEN CONTENT OF FLOUR AND LOAF VOLUME

The wet and dry gluten determination is still used by some chemists. It is argued by the supporters of this test that, in addition to the quantitative feature of the test, a great deal can be learned regarding the quality of the protein or gluten in the wheat or flour sample.

For the purpose of comparing the value of this test with that of the other tests described in this paper dry-gluten determinations were made on 118 samples of hard red winter flour and on 82 samples of hard red spring wheat flour. All the samples were from the 1923 crop.

The data comparing the dry-gluten determinations with the associated loaf volumes are shown in Tables 9 and 10. With the data from the 82 samples of spring wheat flour a coefficient of $+0.2271 \pm 0.0706$ was obtained, whereas the coefficient determined from the data from the 118 samples of the hard red winter flour was $+0.4151 \pm 0.0514$. When the loaf-volume figures were corrected for differences in texture of the crumb, the coefficients changed from $+0.2271 \pm 0.0706$ to $+0.0833 \pm 0.0740$ for the hard red spring wheat flour and from $+0.4151 \pm 0.0514$ to $+0.4181 \pm 0.0512$ for the hard red winter wheat flour.

It would appear from a study of the distribution data and the coefficient of correlation that this determination is of greater value than the kernel-texture determination and almost as good as the crude-protein determination as an indicator of baking quality. The great drawback to this determination, of course, is the difficulty that other laboratories and chemists have in duplicating it. Undeniably differences in the composition of the wash water, the care with which the starch and other adhering material is removed from the dough ball, and the differences in the composition of the flour, which occur when different laboratories extract the flour from the same wheat for the gluten tests, exert far-reaching influences on the results obtained.

As a matter of routine carried on by a single individual at a particular place, the test probably does have its uses, because the operator has the advantage of observing something of the nature of the gluten in the flour sample.

TABLE 9.—*Correlation between percentage of dry gluten and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of dry gluten										
	8–8.99	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	17–17.99	Total
1,900 to 1,999	1		1	4	2	3					11
2,000 to 2,099		3	10	10	3	2	1				29
2,100 to 2,199		1	15	7	5	3	1	1			33
2,200 to 2,299		2	8	9	4	3	1				27
2,300 to 2,399				2	1	2		1			6
2,400 to 2,499				2		3	1	2		1	9
2,500 to 2,599							1	1			2
2,600 to 2,699						1					1
Total	1	6	34	34	15	17	5	5		1	118

r = +0 4151±0 0514.

TABLE 10.—*Correlation between percentage of dry gluten and loaf volume of hard red spring wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of dry gluten						
	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	Total
1,800 to 1,899		1					1
1,900 to 1,999				1			1
2,000 to 2,099	1	2	1	3	1		8
2,100 to 2,199		3	15	11	4	1	34
2,200 to 2,299	1	1	8	5	2		17
2,300 to 2,399		3	4	1	3	2	13
2,400 to 2,499		1		2	4		7
2,500 to 2,599				1			1
Total	2	11	23	24	14	3	82

r = +0.2271±0.0706.

The wet-gluten determination is also sometimes associated with baking quality. It has not come into very general use because of the extreme difficulty of making the test correctly and consistently. Nevertheless, correlation studies were made from the data obtained from 119 samples of flour from the 1923 crop of hard red winter wheat and from 106 samples of the flour milled from the 1923 crop of hard red spring wheat. The results are given in Tables 11 and 12. It will be seen from the data presented that the coefficient obtained from a study of the 119 samples of hard red winter wheat flour was +0.4806 ±0.0475. For the 106 samples of hard red spring wheat flour a small coefficient was obtained, +0.1355 ±0.0643.

TABLE 11.—*Correlation between percentage of wet gluten and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of wet gluten										
	22–24.99	25–27.99	28–30.99	31–33.99	34–36.99	37–39.99	40–42.99	43–45.99	46–48.99	49–51.99	Total
1,800 to 1,899				1							1
1,900 to 1,999	1		2	1	1	2	1				8
2,000 to 2,099	1	5	8	8	1	3	1				27
2,100 to 2,199		2	12	14	4	3	2		1		38
2,200 to 2,299		3	9	5	8	2	2	1			30
2,300 to 2,399					2	1		1			4
2,400 to 2,499				1			3	1	2	1	8
2,500 to 2,599							1		1		2
2,600 to 2,699							1				1
Total	2	10	31	30	16	11	11	3	4	1	119

r=+0.4806±0.0475.

TABLE 12.—*Correlation between percentage of wet gluten and loaf volume of hard red spring wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of wet gluten									
	25–27.99	28–30.99	31–33.99	34–36.99	37–39.99	40–42.99	43–45.99	46–48.99	49–51.99	Total
1,800 to 1,899			1			1				2
1,900 to 1,999			2	1	1		1			5
2,000 to 2,099	1	2	2	3	2		1	1		12
2,100 to 2,199	1	6	17	13	5	2	3	2		49
2,200 to 2,299		2	6	3	5				2	18
2,300 to 2,399		2	4	1	1	4		1		13
2,400 to 2,499					2	4				6
2,500 to 2,599				1						1
Total	2	12	32	22	16	11	5	4	2	106

r=+0.1355±0.0643.

WATER-ABSORBING POWER OF FLOUR AND LOAF VOLUME

The water-absorbing power of flour is important to the baker, as it is usually thought to be a good index of the number of loaves of bread which can be obtained from a given weight of flour.

Correlation studies were therefore made, comparing the water-absorbing power of the flour with the volume of the baked loaf.

Distribution data are given in Tables 13 and 14 for samples of the 1923 hard red winter crop and for samples of the 1924 hard red spring crop. No relationship was found between the water-absorbing power of the flour and the volume of the resulting loaf of bread.

TABLE 13.—*Correlation between water-absorbing power of flour and loaf volume of hard red winter wheat, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of water absorption																	
	53–53.9	54–54.9	55–55.9	56–56.9	57–57.9	58–58.9	59–59.9	60–60.9	61–61.9	62–62.9	63–63.9	64–64.9	65–65.9	66–66.9	67–67.9	68–68.9	69–69.9	Total
1,700 to 1,799								1										1
1,800 to 1,899									1	2	1		1		1			6
1,900 to 1,999	1	1	2		3	2	3	2	2	3				3	3		1	26
2,000 to 2,099		1	1	6	8	6	4	7	2	2	4	4	1			1		47
2,100 to 2,199		2	1	5	6	6	6	6	8	6	1	3						50
2,200 to 2,299		2	2	3	2	2	3	10	6		2	1	2					35
2,300 to 2,399						1	2	1	1	1	1							7
2,400 to 2,499					1			2		2		1	2					8
2,500 to 2,599								1		1								2
2,600 to 2,699									1									1
Total	1	6	6	14	20	17	18	30	21	17	9	9	6	3	4	1	1	183

r = −0.0358 ± 0.0498.

TABLE 14.—*Correlation between water-absorbing power of flour and loaf volume of hard red spring wheat, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of water absorption																
	52–52.9	53–53.9	54–54.9	55–55.9	56–56.9	57–57.9	58–58.9	59–59.9	60–60.9	61–61.9	62–62.9	63–63.9	64–64.9	65–65.9	66–66.9	67–67.9	Total
1,900 to 1,999				1		1	2	1	1		2			1			9
2,000 to 2,099				2		2	3	2		1	1	3	1	1			16
2,100 to 2,199	1		1		1	1	4	7	5	2	2	2	3	1		1	31
2,200 to 2,299		1	2	1	1	1	1	2	2	1	1	3	1	1	1		19
2,300 to 2,399				1		3	1	3			2	1	2	2	1	1	17
2,400 to 2,499							1				1	1	1	1	1		6
2,500 to 2,599			1					1		1	1		1			1	6
2,600 to 2,699						1			1	1	1	1					5
2,700 to 2,799				1				1			1	1					4
2,800 to 2,899					1				1								2
2,900 to 2,999											1						1
3,000 to 3,099													1				1
Total	1	1	4	9	5	11	11	14	15	5	13	10	9	6	2	1	117

r = +0.0341 ± 0.0623.

TABLE 15.—*Correlation between water-holding capacity of flour and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of water-holding capacity																
	66–66.9	67–67.9	68–68.9	69–69.9	70–70.9	71–71.9	72–72.9	73–73.9	74–74.9	75–75.9	76–76.9	77–77.9	78–78.9	79–79.9	80–80.9	81–81.9	Total
1,700 to 1,799								1									1
1,800 to 1,899										2	2		1		1		6
1,900 to 1,999	1	1		1	1	6	3	3	1	1	1			3	3	1	26
2,000 to 2,099			1	2	7	7	7	5	7	1	5	3		1	1		47
2,100 to 2,199			1	3	3	9	8	5	8	5	6	1	1				50
2,200 to 2,299		1	3	2	2	2	4	8	3	5		3	1	1			35
2,300 to 2,399						1	2		1	2	1						7
2,400 to 2,499					1			2		1	1	1	1	1			8
2,500 to 2,599								1			1						2
2,600 to 2,699									1								1
Total	1	2	5	8	14	25	24	21	23	20	16	8	4	6	5	1	183

r = −0.0289 ± 0.0498.

With the 183 samples hard red winter wheat flour samples of the 1923 crop the coefficient was small and negative, namely, −0.0358 ±0.0498. For the 117 samples of spring wheat flour milled from the 1924 crop the coefficient was small and positive, +0.0341 ±0.0623. The 76 flour samples of the 1924 hard red winter crop yielded a negative coefficient of −0.3045 ±0.0702, whereas from the flour milled from the 128 samples of the 1923 grown spring wheat a negative coefficient of −0.0187 ±0.0596 was obtained.

On the possibility that a more significant correlation might result if the water within the flour before the water-absorbing test was made was considered, a new set of correlations was computed, which included this moisture. The data obtained by correlating these new values with the baking tests on the same samples are given in Tables 15 and 16.

TABLE 16.—*Correlation between water-holding capacity of flour and loaf volume of hard red spring wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of water-holding capacity															
	66–66.9	67–67.9	68–68.9	69–69.9	70–70.9	71–71.9	72–72.9	73–73.9	74–74.9	75–75.9	76–76.9	77–77.9	78–78.9	79–79.9	80–80.9	Total
1,900 to 1,999				1	1	1	1	1	1	2				2		10
2,000 to 2,099		1	1	2	2	3	1	2	1		1	1	2		1	18
2,100 to 2,199	1			1		2	5	6	5	2	1	4	1		1	30
2,200 to 2,299		2		1		3	3	2	2	3	3	1	1			21
2,300 to 2,399		1	2	2		3		1	4	2	1	2	1			19
2,400 to 2,499					2	1					1		1			5
2,500 to 2,599							1					1	1			3
2,600 to 2,699									1	2						3
2,700 to 2,799				1			1		1		1					4
2,800 to 2,899									1							1
2,900 to 2,999				1							1					2
3,000 to 3,099													1			1
Total	1	4	4	8	6	13	12	12	16	11	8	11	5	4	2	117

$r = +0.0556 ±0.0622.$

The resulting coefficients are such that no confidence can be placed in this test as an index of gluten quality or of baking strength.

When a comparison is made between the coefficients obtained when the water in the flour was taken into consideration and those obtained when it was not taken into consideration, it is found that the coefficient obtained from the 183 samples of flour of the 1923 winter wheat crop changed from −0.0358 ±0.0498 to −0.0289 ±0.0498; that from the 117 samples of flour milled from the 1924 hard red spring wheat crop changed from +0.0341 ±0.0623 to +0.0556 ±0.0622; that from the 76 samples of the 1924 hard red winter wheat crop changed from −0.3045 ±0.0702 to −0.3134 ±0.0698; and that from the 128 samples of the 1923 hard red spring wheat crop changed from −0.0187 ±0.0596 to −0.0164 ±0.0596.

VISCOSITY OF FLOUR IN WATER SUSPENSIONS AS AN INDEX OF GLUTEN QUALITY

Stimulated by the earlier researches of Weaver and Goldtrap (*25*) and as a direct result of the investigations of Gortner and Sharp (*17*), several viscometric methods for measuring the gluten quality have come into prominence. In the Gortner and Sharp method (*17*), gluten quality is measured by determining the slope of the curve formed by plotting the logarithm of the viscosities as ordinates against

the logarithm of the flour concentration as abscissas. When this is done, it is found that the values lie on a straight line. The increment of increase of the logarithm of the viscosity per increment of increase of the logarithm of flour concentration determines the slope of this line. The equation is log. viscosity $= a - b$ (log. concentration) where a and b are constants. The position of the line on the viscosity axis at zero flour concentration is determined by a, and b is the tangent of the angle which the line makes with the horizontal. Thus b is directly related to the slope of the line and may be taken as a numerical value of gluten quality. The value b may be measured from the plotted line or computed by the method of least squares (*14*).

As the method prescribed by Gortner and Sharp (*17*) was considered too long and involved for rapid laboratory or control work, numerous short cuts and modifications of it have been tried, with varying success. Investigations of this kind have been reported from time to time in such papers as those of Morgan (*16*), Blish and Sandstedt (*4*), Smith (*22*), and Durham (*6*).

In all these investigations a determination of the viscosity of one concentration of flour or wheat, as the case might be, received the most attention. Some investigators washed the flour or wheat free from electrolytes, whereas others ran their tests on the sample without washing it.

Fundamentally, the one-concentration idea is unsound, as has been pointed out by Gortner (*9*), but for that matter the whole principle of measuring a plastic material like flour with a torsional viscosimeter, such as the MacMichael (*10, p. 349*), is subject to criticism.

Nevertheless, preliminary studies were made of the same samples of the 1923 hard red winter and hard red spring wheat flour that had been studied before. The one-concentration plan was used, namely, washing 20 gm. of flour free from electrolytes with 2,000 c. c. of distilled water and then making it to a 100 c. c. volume.

The MacMichael viscosimeter (*11*) was used, in which were employed a No. 30 wire, a 2 c. c. bob, and a cup making 76 turns per minute. Suspensions were poured into the cup of the viscosimeter until the 2 cm. mark on the bob was reached.

Normal lactic acid was added to each flour-and-water suspension until each flour was the same in respect to hydrogen-ion concentration.

The data from this study are given in Tables 17 and 18.

- TABLE 17.—*Correlation between viscosity of one concentration of flour and water and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume, in cubic centimeters	Number of samples according to viscosity in degrees MacMichael																To tal
	100-124	125-149	150-174	175-199	200-224	225-249	250-274	275-299	300-324	325-349	350-374	375-399	400-424	425-449	450-474	475-499	
1,700 to 1,799		1															1
1,800 to 1,899					2	3	1										6
1,900 to 1,999	1	1	4	5	2	5	3	2			2				1		26
2,000 to 2,099	1	4	6	12	11	4	3	1	1		2	1	1				47
2,100 to 2,199			5	6	18	12	3	1	3	1	1						50
2,200 to 2,299	1	2		9	6	8	7	2									35
2,300 to 2,399					1	3	2	1									7
2,400 to 2,499					1	1		2	4								8
2,500 to 2,599									1	1							2
2,600 to 2,699							1										1
Total	3	8	15	32	41	36	20	9	9	2	5	1	1			1	183

r = .87±0.0481.

TABLE 18.—*Correlation between viscosity of one concentration of flour and water and loaf volume of hard red spring wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to viscosity in degrees MacMichael											
	125–149	150–174	175–199	200–224	225–249	250–274	275–299	300–324	325–349	350–374	375–399	Total
1,800 to 1,899		1		1		1						3
1,900 to 1,999			2	3	2			1				8
2,000 to 2,099	2			6	1	2	2					13
2,100 to 2,199		2	2	9	12	6	7	8	1			47
2,200 to 2,299			2	3	7	5	4	3				24
2,300 to 2,399			2	3		5	2	3		1	1	17
2,400 to 2,499					1	7			1			9
2,500 to 2,599				1		1	1	1				4
2,600 to 2,699						1		1				2
2,700 to 2,799							1					1
Total	2	3	8	26	23	28	17	17	2	1	1	128

$r = +0.3260 \pm 0.0533$.

It will be seen from an examination of Table 18 that there was a significant relationship existing between the one concentration or absolute viscosity of the flour-and-water suspension of the hard red spring wheat flour under the same conditions of hydrogen-ion concentration and the baking strength as interpreted by loaf volume. The coefficient in this series of samples was $+0.3260 \pm 0.0533$.

Although the coefficient obtained with the hard red winter wheat samples was not large, namely, $+0.1887 \pm 0.0481$, it is likewise significant, inasmuch as it is almost four times the probable error.

When differences in the texture of the various loaves of bread were taken into consideration these coefficients changed somewhat. The samples of winter wheat flour gave a coefficient of $+0.1643 \pm 0.0485$, as compared with $+0.1887 \pm 0.0481$, whereas for samples of spring wheat flour the coefficient changed from $+0.3260 \pm 0.0533$ to $+0.3384 \pm 0.0528$.

These coefficients are larger than those obtained when texture of kernel was taken as an index of gluten quality, and they are decidedly larger than the coefficients obtained when the water-absorbing power of the flours was studied as an index of gluten quality. They are, however, much lower in value than the coefficients obtained from a study of either the protein content of the wheat flour as indices of gluten qual. ·.

Because of the encouraging results obtained with the one concentration of flour and water on the samples of the 1923 crop, the viscometric study was enlarged when applied to the 1924 crop. On the samples for that crop, in addition to the one-concentration test the quality angle *b* of Gortner and Sharp (*17*), the viscosity of 2 gm. of protein, and the viscosity of 1 per cent of protein were compared with the associated loaf volumes. The only change in the technique as described above was that the flour-and-water suspension was acidified by means of one-half c. c. of 20 per cent lactic acid, according to the recommendations of Gortner (*9*). Tests were made by the four methods mentioned above on 116 samples of hard red spring wheat and 76 samples of hard red winter wheat of the crop of 1924.

As before, when the one-concentration method was used with the hard red spring flour a significant correlation of $+0.3310 \pm 0.0558$

was obtained, this coefficient changing to $+0.2112 \pm 0.0598$ when the texture of the crumb was taken into account. Again, as with the 1923 crop, only a slight relationship existed between the one-concentration viscosity values of the 76 samples of hard red winter wheat and the volume of the baked loaf, the coefficient of correlation being $+0.0745 \pm 0.0774$. Neither of the above coefficients was improved upon by being corrected for the texture of the crumb.

ANGLE *b* AS AN INDEX OF BAKING STRENGTH

A comparison of angle *b* with loaf volume as determined on 114 samples of the 1924 hard red spring wheat crop and on 76 samples of the 1924 hard red winter wheat crop are given in Tables 19 and 20.

From the 114 samples of hard red spring wheat flour, little relationship was apparent when angle *b* of Gortner was determined and the values correlated with the associated loaf volume, the coefficient of correlation being -0.0320 ± 0.0631. For the 76 samples of hard red winter wheat flour a fairly high coefficient was found when angle *b* was compared with the associated loaf volume, namely, $+0.2214 \pm 0.0736$.

TABLE 19.—*Correlation between quality angle b and loaf volume of hard red spring wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to range in tangent of Gortner angle b											
	1.30-1.44	1.45-1.59	1 60-1.74	1.75-1.89	1.90-2.04	2.05-2.19	2.20-2.34	2.35-2.49	2.50-2.64	2.65-2.79	2.80-2.94	Total
1,900 to 1,999			2	5		1	1					9
2,000 to 2,099		1	3	2	3	1	1	2		1		14
2,100 to 2,199	1	1	4	4	7	7	3	1	1	1		30
2,200 to 2,299	1		1	1	3	6	3	1	2			18
2,300 to 2,399		2	1	4	2	1	5	1	1		1	18
2,400 to 2,499	1			2		1		1		1		6
2,500 to 2,599			2		1	1	1	1				6
2,600 to 2,699	1	1		1	1		1					5
2,700 to 2,799	1			1	1			1				4
2,800 to 2,899	1				1							2
2,900 to 2,999									1			1
3,000 to 3,099				1								1
Total	6	5	13	21	20	17	16	7	5	3	1	114

r=−0.0320±0.0631.

TABLE 20.—*Correlation between quality angle b and loaf volume of hard red winter wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to range in tangent of Gortner angle b												
	1.60-1.74	1.75-1.89	1.90-2.04	2.05-2.19	2.20-2.34	2.35-2.49	2.50-2.64	2.65-2.79	2.80-2.94	2.95-3.09	3.10-3.24	3.25 and more	Total
1,600 to 1,699				1							1		2
1,700 to 1,799		1	1										2
1,800 to 1,899	5	2	3	1	2								13
1,900 to 1,999		4	6	1	2	1	1			1		1	17
2,000 to 2,099	2	2	2	1	3		1				2		13
2,100 to 2,199		3	1	5	2	2	1						15
2,200 to 2,299				2	2	2			1	1			8
2,300 to 2,399						1				1			2
2,400 to 2,499			1										1
2,500 to 2,599				2		1							3
Total	7	12	14	13	11	6	4	1	1	3	3	1	76

r=+0.2214±0.0736.

VISCOSITY OF 2 GRAMS OF PROTEIN AND LOAF VOLUME

Since it has been shown that flour starch under the conditions of the Gortner and Sharp test (*17*) has but little influence upon the viscosity, it has been suggested that when the total protein content of the flour sample is known, single concentrations of flour could be prepared in such a way as to make possible viscometric measurements on the same amount of protein. This was done with 113 samples of hard red spring wheat flour and with 73 samples of hard red winter wheat flour of the 1924 crop. No distribution tables are given. Suffice it to say that the coefficient of correlation obtained by comparing the 73 samples of hard red winter wheat flour with the associated loaf volume was +0.0409 ±0.0788, whereas from the 113 samples of hard red spring wheat flour a coefficient of −0.0251 ±0.0634 was obtained.

VISCOSITY OF 1 PER CENT OF PROTEIN AND LOAF VOLUME

It has likewise been suggested that viscosity readings may be reduced to a common protein basis by dividing the viscosity readings by the protein content of the flour. Coefficients of correlation resulting from a comparison of such values with the associated baking tests were of no practical significance.

OTHER CORRELATION STUDIES

In addition to the correlations measuring gluten quality, the relationships existing between the physical and chemical factors were compared. Texture of wheat, protein content of wheat, water-absorbing power of flour, and viscosity determinations in their various forms were compared with each other for each class of wheat for the two crop years. These data are given in Tables 21 and 22.

The relationship existing between texture of the kernel; that is, the percentage of dark, hard, and vitreous kernels in the wheat, and other tests was first considered.

The degree of relationship found between the protein content of the wheat and kernel texture was high. In each crop year and in both classes of wheat there were also fairly significant relationships existing between the texture of kernel and the water-absorbing power of the flour and between the texture of the kernel and the viscosity of one concentration of flour and water. The coefficient from the latter determination was just as high in most instances as was the coefficient of correlation between the kernel texture and the protein content of the wheat. When the Gortner angle *b* (*9*) was considered, the relationship between it and the texture of the kernel was small.

The protein content of the flour was next compared with the water-absorbing power of the flour, with the viscosity of one concentration of flour and water, with the Gortner angle *b*, and with the percentage of wet and dry gluten in the flour.

TABLE 21.—*Correlations between the kernel texture of wheat and other tests used to express gluten quality*

Tests	Number of samples	Percentage of dark, hard, and vitreous kernels			Comparative test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
1923:									
Hard red winter wheat—									
Texture of wheat and protein content	183	67.5	99.9	6.0	a 12.0	a 15.90	a 8.20	+0.6004	±0.0319
Texture of wheat and water-absorbing power	183	67.5	99.9	6.0	a 72.9	a 81.70	a 66.90	+.4190	±.0411
Texture of wheat and viscosity of one concentration of flour and water	183	67.5	99.9	6.0	b 226	b 476	b 108	+.6776	±.0270
Hard red spring wheat—									
Texture of wheat and protein content	128	81.0	100.0	25.2	a 12.65	a 15.20	a 9.20	+.6414	±.0351
Texture of wheat and water-absorbing power	128	81.0	100.0	25.2	a 71.70	a 84.00	a 64.80	+.3928	±.0504
Texture of wheat and viscosity of one concentration of flour and water	128	81.0	100.0	25.2	b 252	b 388	b 128	+.5597	±.0409
1924:									
Hard red winter wheat—									
Texture of wheat and protein content	76	78.4	100.0	18.2	a 12.90	a 18.00	a 8.40	+.5593	±.0582
Texture of wheat and water-absorbing power	76	78.4	100.0	18.2	a 73.60	a 80.80	a 66.60	+.4605	±.0610
Texture of wheat and viscosity of one concentration of flour and water	75	78.1	100.0	18.2	b 208	b 417	b 72	+.3166	±.0701
Texture of wheat and Gortner angle b	76	78.4	100.0	18.2	c 2.18	c 3.69	c 1.64	−.2065	±.0741
Hard red spring wheat—									
Texture of wheat and protein content	113	89.4	99.9	18.0	a 13.70	a 18.80	a 9.50	+.3981	±.0536
Texture of wheat and water-absorbing power	116	89.8	99.9	18.0	a 73.80	a 81.00	a 66.80	+.3824	±.0535
Texture of wheat and viscosity of one concentration of flour and water	113	89.5	99.9	18.0	b 298	b 423	b 98	+.3537	±.0555
Texture of wheat and Gortner angle b	116	89.8	99.9	18.0	c 2.00	c 2.81	c 1.27	+.0102	±.0626

a Per cent.　　b Degrees MacMichael.　　c Tangent of angle b.

TABLE 22.—*Correlations between various tests used to express gluten quality*

Tests	Crop year	Class of wheat	Number of samples	Percentage of independent factor			Dependent-factor determinations			Coefficient of correlation	Probable error
				Average	Maximum	Minimum	Average	Maximum	Minimum		
Protein content of flour and viscosity of one concentration of flour and water	1923	Hard red winter	183	10.89	15.68	7.30	226[a]	476[a]	108[a]	+0.7449	±0.0222
Do	1923	Hard red spring	128	11.71	15.10	8.32	252[a]	388[a]	128[a]	+.7344	±.0275
Do	1924	Hard red winter	116	12.99	17.29	8.47	238[a]	423[a]	98[a]	+.5307	±.0450
Do	1924	Hard red spring	75	11.80	17.10	6.78	208[a]	417[a]	72[a]	+.0297	±.0470
Protein content of flour and water-holding capacity of flour	1923	Hard red winter	183	10.89	15.68	7.30	72.9[b]	81.7[b]	66.9[b]	+.4327	±.0450
Do	1923	Hard red spring	128	11.71	15.10	8.32	71.7[b]	84.0[b]	65.6[b]	+.1381	±.0585
Do	1924	--do--	117	12.99	17.29	8.47	73.8[b]	81.0[b]	66.8[b]	+.4293	±.0509
Do	1924	--do--	76	11.80	18.00	6.78	73.6[b]	80.8[b]	66.6[b]	+.5813	±.0512
Protein content of wheat and Gortner angle b	1924	Hard red winter	76	12.99	18.00	8.40	2.18[c]	3.69[c]	1.64[c]	-.3704	±.0664
Do	1924	Hard red spring	116	13.79	18.80	9.50	2.00[c]	2.81[c]	1.27[c]	+.0512	±.0625
Protein content of wheat flour and Gortner angle b	1924	Hard red winter	71	11.49	16.70	7.52	2.21[c]	3.69[c]	1.64[c]	+.1584	±.0780
Do	1924	Hard red spring	121	12.88	17.29	8.47	2.00[c]	2.80[c]	1.27[c]	-.0150	±.0613
Water-holding capacity and Gortner angle b	1924	Hard red winter	76	73.60	80.80	66.60	2.18[c]	3.69[c]	1.64[c]	-.3863	±.0658
Do	1924	Hard red spring	117	73.80	81.00	66.80	2.00[c]	2.81[c]	1.27[c]	+.1020	±.0617
Water-holding capacity and viscosity of one concentration of flour and water	1923	Hard red winter	183	72.90	81.70	66.90	226[a]	476[a]	108[a]	+.5620	±.0341
Do	1923	Hard red spring	128	72.90	81.70	66.90	252[a]	388[a]	128[a]	+.1739	±.0578
Do	1924	Hard red winter	75	73.80	81.00	66.60	208[a]	423[a]	72[a]	+.5593	±.0635
Do	1924	Hard red spring	116	73.80	81.00	66.80	228[a]	423[a]	98[a]	+.3267	±.0559
Percentage of wet gluten and protein content of flour	1923	--do--	106	12.24	15.50	9.80	33.71[b]	49.36[b]	25.10[b]	+.8643	±.0166
Do	1923	Hard red winter	119	11.04	15.68	8.61	33.78[b]	52.12[b]	22.18[b]	+.8988	±.0119
Do	1923	--do--	118	12.37	16.79	9.82	11.87[b]	17.29[b]	8.52[b]	+.8920	±.0127
Protein content of wheat and percentage of dry gluten	1923	Hard red spring	82	12.79	14.95	10.84	13.00[b]	15.76[b]	10.27[b]	+.7551	±.0320
Protein content of flour and percentage of wet gluten	1923	Hard red winter	119	11.04	15.68	8.61	33.78[b]	52.12[b]	22.18[b]	+.8988	±.0119
Do	1923	Hard red spring	106	12.13	14.65	8.80	35.71[b]	49.36[b]	25.10[b]	+.8643	±.0166
Protein content of flour and percentage of dry gluten	1923	Hard red winter	118	11.24	14.25	8.64	11.89[b]	17.29[b]	8.58[b]	+.9166	±.0099
Do	1923	Hard red spring	82	12.13	14.25	9.80	13.00[b]	15.76[b]	10.27[b]	+.8473	±.0210
Percentage of wet gluten and viscosity of one concentration of flour and water	1923	--do--	106	35.71	49.36	25.10	245[a]	346[a]	151[a]	+.1860	±.0633
Do	1923	Hard red winter	119	33.78	52.12	22.18	222[a]	373[a]	108[a]	+.7451	±.0275
Protein content of wheat and protein content of flour	1923	Hard red spring	128	12.65	15.20	9.20	11.71[b]	15.10[b]	8.32[b]	+.9388	±.0071

[a] Degrees MacMichael.   [b] Per cent.   [c] Tangent of angle b.

A high and positive correlation was found between the protein content of the flour and the water absorption in every case with the exception of spring wheat flours of the 1923 crop. There was also a high and positive correlation between the protein content of the flour and the absolute or one concentration viscosity test for both classes of wheat in each crop year. When flour protein was compared with angle *b* the relationship was low with the hard red winter wheat crop of 1924 and of no significance with the samples of flour from the 1924 hard red spring-wheat crop.

The correlation between the protein of flour and the percentage of dry gluten was almost perfect. A coefficient of $+0.9166 \pm 0.0099$ was obtained from the 1923 hard red winter wheat flour and one of $+0.8473 \pm 0.0210$ for 82 samples of flour of the 1923 hard red spring wheat crop. High coefficients were also obtained when the protein of the flour was compared with the percentage of wet gluten in the same samples tested for dry gluten content, $+0.8988 \pm 0.0119$ being the coefficient obtained from a study of the 119 samples of hard red winter flour and $+0.8643 \pm 0.0166$, that for the hard red spring wheat flour.

The relation existing between gluten quality angle *b* and the protein content of the wheat is of no significance, nor is the relationship found between the water-absorbing power of the flour and quality angle *b*.

In three out of four instances a high and significant relation was found between the absolute one-concentration test and the water-absorbing power of the flour.

High correlation coefficients were likewise found between the protein content of the wheat and the texture of the kernel, between the protein content of the flour and the water-absorbing power of the flour, between the viscosity of a single concentration of the flour and water and the protein of the flour, and between the percentage of wet and dry gluten and the protein content of the flour.

The substitution of one-concentration viscosity values for the protein in the flour resulted in a coefficient somewhat lower in most instances than that found to exist between the protein content of flour and the associated loaf volume. Thus it becomes apparent from a study of the distribution tables that the one-concentration viscosity test is a measure of the quantity of protein in wheat flour rather than an index of the gluten quality, if the baking test is used as the measure of gluten quality.

Why higher correlation values were not forthcoming, especially from the use of some of the well-known tests, such as the water-absorbing power of the flour or kernel texture of the wheat, it is difficult to say. Gortner (*9*) criticizes baking technique in general because the majority of baking experiments are not so conducted as to insure the presence of an adequate amount of diastase in all instances. Alsberg (*1*) objects to the use of only one baking method for studying the gluten quality of wheat flour, arguing that several trials should be made with the same flour under different baking conditions before final judgment is passed on the baking quality of the flour. Possibly if an adequate supply of diastase was at hand or if several bakings were made, using different formulas, a different set of coefficients would be obtained. Blish (*4*), however, baked

flours with adequate diastatic qualities and found no answer to the gluten-quality problem even when this factor was considered.

On the other hand, tests made in connection with other investigations show that a change in baking procedure will affect the correlations. Eighty-four samples of the 1923 hard red winter wheat flour and 39 samples of the 1923 hard red spring wheat flour were baked in another laboratory and by another baker. The results obtained by this outside laboratory were compared with the bakings made of the identical flours in the milling and baking laboratory of the Bureau of Agricultural Economics. The baking data from each laboratory were compared with the protein content of the flour, and the following correlation coefficients were obtained. With the 39 samples of hard red spring wheat the coefficient of correlation obtained from the data submitted by the outside laboratory was + 0.2757 ± 0.0998, whereas from the data submitted by the milling and baking laboratory the coefficient was + 0.4405 ± 0.0870. A comparison of the baking data of the 84 samples of hard red winter wheat flour with their crude-protein contents showed a coefficient of + 0.3357 ± 0.0653 in the work of the outside laboratory and one of + 0.3974 ± 0.0620 in the milling and baking laboratory.

It is apparent, then, that before a set of results can be obtained that will be standard for all laboratories, the recommendation of Fitz (7) relative to a standard baking test will have to be seriously considered.

In Tables 23, 24, 25, and 26 is given a summary of the coefficients obtained from the various tests for gluten quality.

TABLE 23.—*Correlations between loaf volume and physical and chemical tests of gluten quality of hard red winter wheat flours, crop of 1923*

TESTS IN WHICH DETERMINATIONS WERE NOT CORRECTED FOR CRUMB TEXTURE

Test	Num-ber of sam-ples	Loaf volume, c. c.			Quality test deter-minations			Coeffi-cient of corre-lation	Prob-able error
		Aver-age	Maxi-mum	Mini-mum	Aver-age	Maxi-mum	Mini-mum		
Kernel texture of wheat	183	2, 129	2, 695	1, 770	a 67. 5	a 99. 9	a 6. 00	+0. 0990	±0. 0494
Protein content of wheat	183	2, 129	2, 695	1, 770	a 12. 00	a 15. 90	a 8. 20	+. 4281	±. 0407
Protein content of flour	183	2, 129	2, 695	1, 770	a 10. 89	a 15. 68	a 7. 30	+. 4249	±. 0409
Percentage of dry gluten in flour	118	2, 169	2, 695	1, 885	a 11. 89	a 17. 29	a 8. 53	+. 4151	±. 0511
Percentage of wet gluten in flour	119	2, 169	2, 695	1, 885	a 33. 78	a 52. 12	a 22. 18	+. 4806	±. 0475
Water-absorbing power of flour	183	2, 129	2, 695	1, 770	b 60. 1	b 74. 7	b 53. 5	−. 0358	±. 0498
Water-holding capacity of flour	183	2, 129	2, 695	1, 770	b 72. 9	b 81. 7	b 66. 9	−. 0289	±. 0498
Viscosity of 1 concentration of flour and water	183	2, 129	2, 695	1, 770	c226	c476	c108	+. 1887	±. 0481

TESTS IN WHICH DETERMINATIONS WERE CORRECTED FOR CRUMB TEXTURE

Test	Num-ber of sam-ples	Loaf volume, c. c.			Quality test deter-minations			Coeffi-cient of corre-lation	Prob-able error
Kernel texture of wheat	183	1, 913	2, 533	1, 561	a 67. 5	a 99. 9	a 6. 00	+0. 1027	±0. 0493
Protein content of wheat	183	1, 913	2, 533	1, 561	a 12. 00	a 15. 90	a 8. 20	+. 3932	±. 0422
Portein content of flour	183	1, 913	2, 533	1, 561	a 10. 89	a 15. 68	a 7. 30	+. 3816	±. 0426
Percentage of dry gluten in flour	118	1, 953	2, 533	1, 649	a 11. 89	a 17. 29	a 8. 58	+ 4181	±. 0512
Percentage of wet gluten in flour	119	1, 953	2, 533	1, 649	a 33. 78	a 52. 12	a 22. 18	+. 4792	±. 0476
Water-holding capacity of flour	183	1, 913	2, 533	1, 561	b 72. 9	b 81. 7	b 66. 9	−. 0711	±. 0496
Viscosity of 1 concentration of flour and water	183	1, 913	2, 533	1, 561	c226	c476	c108	+. 1643	±. 0485

a Per cent.   b Cubic centimeters.   c Degrees MacMichael.

TABLE 24.—*Correlations between loaf volume and physical and chemical tests of gluten quality of hard red spring wheat flours, crop of 1923*

TESTS IN WHICH DETERMINATIONS WERE NOT CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
Kernel texture of wheat	128	2,268	2,725	1,830	a 81.00	a 100.00	a 25.20	+0.2612	±0.0556
Protein content of wheat	128	2,268	2,725	1,830	a 12.65	a 15.20	a 9.20	+.4610	±.0470
Protein content of flour	128	2,268	2,725	1,830	a 11.71	a 15.10	a 8.32	+.4641	±.0468
Percentage of dry gluten in flour	82	2,212	2,500	1,870	a 13.00	a 15.76	a 10.27	+.2271	±.0706
Percentage of wet gluten in flour	106	2,183	2,525	1,840	a 35.71	a 49.36	a 25.10	+.1355	±.0643
Water-absorbing power of flour	128	2,268	2,725	1,830	b 58.60	b 71.50	b 52.90	+.0187	±.0596
Water-holding capacity of flour	128	2,268	2,725	1,830	b 71.10	b 84.00	b 64.80	−.0164	±.0596
Viscosity of 1 concentration of flour and water	128	2,268	2,725	1,830	c 252	c 388	c 128	+.3260	±.0533

TESTS IN WHICH DETERMINATIONS WERE CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
Kernel texture of wheat	128	1,957	2,540	1,624	a 81.00	a 100.00	a 25.20	+0.2937	±0.0545
Protein content of wheat	128	1,957	2,540	1,624	a 12.65	a 15.20	a 9.20	+.4068	±.0498
Protein content of flour	128	1,957	2,540	1,624	a 11.71	a 15.10	a 8.32	+.3856	±.0508
Percentage of dry gluten in flour	82	1,962	2,250	1,710	a 13.00	a 15.76	a 10.27	+.0822	±.0740
Percentage of wet gluten in flour	106	1,947	2,280	1,656	a 35.71	a 49.36	a 25.10	+.1577	±.0639
Water-holding capacity of flour	128	1,957	2,540	1,624	b 71.10	b 84.00	b 64.80	+.0965	±.0591
Viscosity of 1 concentration of flour and water	128	1,957	2,540	1,624	c 252	c 388	c 128	+.3384	+.0528

a Per cent. b Cubic centimeters. c Degrees MacMichael.

TABLE 25.—*Correlations between loaf volume and physical and chemical tests for gluten quality of hard red spring wheat flours, crop of 1924*

TESTS IN WHICH DETERMINATIONS WERE NOT CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
Kernel texture of wheat	113	2,270	2,970	1,920	a 89.50	a 99.90	a 18.00	+0.1517	±0.0620
Protein content of wheat	116	2,286	2,970	1,920	a 13.70	a 18.80	a 9.50	+.4184	±.0517
Protein content of flour	116	2,286	2,970	1,920	a 12.99	a 17.29	a 8.47	+.4005	±.0526
Water-absorbing power of flour	117	2,286	2,970	1,920	b 60.20	b 67.40	b 52.90	+.0341	±.0623
Water-holding capacity of flour	117	2,286	2,970	1,920	b 73.80	b 81.00	b 66.80	+.0556	±.0622
Viscosity of 1 concentration of flour and water	116	2,286	2,970	1,920	c 238	c 423	c 98	+.3310	±.0558
Viscosity of 2 grams of protein	113	2,042	2,580	1,640	c 146	c 277	c 52	−.0070	±.0635
Viscosity, Gortner angle b	114	2,270	2,970	1,920	d 2.00	d 2.81	d 1.27	−.0320	±.0631

TESTS IN WHICH DETERMINATIONS WERE CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
Kernel texture of wheat	113	2,044	2,909	1,418	a 89.50	a 99.90	a 18.00	+0.1249	±0.0625
Protein content of wheat	116	2,039	2,909	1,418	a 13.70	a 18.80	a 9.50	+.3694	±.0546
Protein content of flour	116	2,039	2,909	1,418	a 12.99	a 17.29	a 8.47	+.3278	±.0559
Water-holding capacity of flour	117	2,039	2,909	1,418	b 73.80	b 81.00	b 66.80	+.1076	±.0616
Viscosity of 1 concentration of flour and water	116	2,039	2,909	1,418	c 238	c 423	c 98	+.2112	±.0598
Viscosity of 2 grams of protein	113	1,806	2,394	920	c 146	c 277	c 52	−.0251	±.0634
Viscosity, Gortner angle b	114	2,044	2,909	1,418	d 2.00	d 2.81	d 1.27	−.0277	±.0631

a Per cent. b Cubic centimeters. c Degrees MacMichael. d Tangent of the angle b.

TABLE 26.—*Correlations between loaf volume and physical and chemical tests of gluten quality of hard red winter wheat flours, crop of 1924*

TESTS IN WHICH DETERMINATIONS WERE NOT CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
Kernel texture of wheat	76	2,041	2,580	1,640	a 78.4	a100.00	a 18.20	+0.0287	±0.0773
Protein content of wheat	76	2,041	2,580	1,640	a 12.90	a 18.00	a 8.40	−.0370	±.0773
Protein content of flour	76	2,041	2,580	1,640	a 11.80	a 17.10	a 6.78	+.1153	±.0763
Water-absorbing power of flour	76	2,041	2,580	1,640	b 60.20	67.70	b 52.90	−.3045	±.0702
Water-holding capacity of flour	76	2,041	2,580	1,640	b 73.60	b 80.80	b 66.60	−.3134	±.0698
Viscosity of 1 concentration	76	2,041	2,580	1,640	c208	c417	c 72	+.0745	±.0774
Viscosity of 2-gram sample	73	2,260	3,030	1,920	c135	c262	c 58	+.0409	±.0788
Viscosity, Gortner angle b	76	2,041	2,580	1,640	d 2.18	d 3.69	d 1.64	+.2214	±.0736

TESTS IN WHICH DETERMINATIONS WERE CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
Kernel texture of wheat	76	1,802	2,394	927	a 78.40	a100.00	a 18.20	+0.0328	±0.0773
Protein content of wheat	76	1,802	2,394	927	a 12.90	a 18.00	a 8.40	−.0353	±.0773
Protein content of flour	76	1,802	2,394	927	a 11.80	a 17.10	a 6.78	+.0464	±.0772
Water-holding capacity of flour	76	1,802	2,394	927	b 73.60	b 80.60	b 66.60	−.2613	±.0721
Viscosity of 1 concentration	76	1,802	2,394	927	c208	c417	c 72	+.0550	±.0777
Viscosity of 2-gram sample	73	2,029	2,909	1,418	c135	c262	c 58	−.0377	±.0778
Viscosity, Gortner angle b	76	1,802	2,394	927	d 2.18	d 3.69	d 1.64	+.1246	±.0736

a Per cent.   b Cubic centimeters.   c Degrees MacMichael.   d Tangent of angle b.

CONCLUSIONS

Although there were slight differences in the order of merit, dependent on the class of wheat and the crop year, it appears from the data submitted that a crude-protein determination, either on the wheat or on the flour milled from this wheat, is the best single-factor test of gluten quality.

Next in order of merit comes the washed-gluten test. This test gives remarkably good results in the hands of a single operator, but the results obtained are not easily reproducible. This is not true of the crude-protein test.

Among the newer viscosity tests for determining baking strength, the single-concentration test is the only one that has given any satisfaction. Compared with the other tests reported in this investigation, this type of viscosity test is nearly as good as the dry-gluten or wet-gluten test and decidedly better than a determination of kernel texture or a determination of the water-absorbing power of the flour.

Tests of the kernel texture and the water-absorbing power of flour did not give very high coefficients of correlation. These tests rank as about equal in choice as tests for gluten quality.

As a measure of baking strength, the four-point system of Gortner and Sharp (*17*), from which the angle b (*9*) is calculated, has proved of negative value as a means of predicting baking strength. The same is true of tests of the viscosity of an even amount of protein or the viscosity of an even percentage of protein as a measure of gluten quality.

The lack of closer agreement between the results of the newer gluten-quality tests and the volume of the baked loaf is, no doubt, partly due to variations in baking procedure.

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THE LIFE HISTORY, HABITS, AND ECONOMIC IMPORTANCE OF SOME MONONCHS[1]

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INTRODUCTION

The relationships existing, under actual field conditions, between the predacious nemas of the genus Mononchus and the economically important plant-parasitic nemas is a subject upon which published information is exceedingly meager. There is likewise but little information concerning the factors which may influence these relationships. In view of these facts, the studies here described were begun for the purpose of learning, if possible, what mononchs were present in the soils of Utah and Idaho fields infested with the sugar-beet nema, *Heterodera schachtii* Schmidt, and what the possibilities were of their being, or becoming, of economic importance in the control of this highly destructive pest.

At the same time it was imperative that data be collected on the factors which might influence the mononchs in their habits and thus directly or indirectly have a bearing on the number in the soil. The factors immediately suggested were: Temperature, moisture, food supply, and diseases or enemies. Doubtless these are the four most important factors in nemic life, any one of which may vitally influence the activities and number of mononchs.

METHODS

Mononch colonies were located by making about 200 examinations of soil from fields infested with *Heterodera schachtii*. These fields were distributed over practically all the sugar-beet growing sections of Utah and southern Idaho. The soils examined varied from the heavy clay loams of the lower-valley fields to the light sandy soils of the upper-valley levels. From these examinations it was possible to obtain fairly accurate data on the species of mononchs present and the numbers in which they occurred in the various fields.

The soil samples were examined in the laboratory, using the methods devised by Cobb.[3] It was found that the mononch population of the fields varied greatly. In some of the heavy clay loam soils no mononchs were found, while in the lighter sandy loams as many as four species were present. The populations of the various species ranged from only a few specimens to nearly 300,000,000 per acre.

In 1923 two fields were selected which apparently offered the most favorable prospects because of the number of mononchs present and the degree of *Heterodera schachtii* infestation. These two

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 3
Feb. 1, 1927
Key No. G-555

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fields contained colonies of all the species of mononchs found in the cultivated areas of Utah, making it possible to obtain a maximum amount of data with a minimum of labor.

To determine if there was any increase in the mononch population correlated with the numbers of *Heterodera schachtii* present, arrangements were made with the owners of these fields to plant the latter with sugar beets every year. This arrangement insured a large population of *H. schachtii*, upon which the mononchs could feed if they were inclined to do so.

DESCRIPTION OF FIELDS SELECTED

FIELD NO. 1—MARION BULLEN FARM, LEWISTON, UTAH

Sugar beets had been grown in the field on the Bullen farm almost continuously for about 20 years, and it had become so severely infested with *Heterodera schachtii* that the crop was practically a failure. The soil is a light sandy loam of loose texture, typical of the Lewiston section. In general, the soil is run down because of overcropping with beets and lack of manure. It is irrigated by the "subbing" method; that is, by making large ditches 50 to 100 feet apart, which are kept filled with water until the intervening spaces are completely saturated. However, this field lies alongside a large canal, and it was not necessary to apply any water in addition to that which leached into it from the canal. This condition, it might be expected, would give a more uniform distribution of moisture from month to month than would be found in a field irrigated by the furrow method, where the sudden application of water would quickly saturate the upper soil but would be followed by almost immediate drying out.

Two mononchs were found inhabiting this field: *Mononchus macrostoma* Bastian, 1866, and *M. parabrachyurus* Thorne, 1924.

FIELD NO. 2—A. W. DAVIS FARM, SALEM, UTAH

The field on the Davis farm has a medium sandy loam soil, is well drained, and is kept in excellent condition by cultivation and application of barnyard manure. Sugar beets have been grown on it continuously for about 15 years, but the infestation of *Heterodera schachtii* did not appear until about 1919. It is irrigated by the usual furrow method, and the water supply is sufficient to allow an application whenever needed.

Four mononchs were found in this field: *Mononchus papillatus* Bastian, 1866; *M. sigmaturus* Cobb, 1917; *M. macrostoma* Bastian, 1866; and *M. parabrachyurus* Thorne, 1924. Of these *M. parabrachyurus* was not found in 1923, and *M. macrostoma* occurred very rarely.

STUDY OF THE NEMA POPULATION

Soil samples were taken to a depth of 2 feet during 1923, but so few nemas were found in the lowest 6 inches that the following years the samples were taken to a depth of only 18 inches. The soil was collected by the Cobb method, in tubes having an area of one-millionth of an acre. Each sample examined was an aliquot part of from two to six samples, usually two. The soil of each 2-inch depth was col-

lected separately, care being exercised not to mix it with soil from above or below the specified depth.

Samples were collected at intervals which, unfortunately, were not of any definite length of time, as other work interfered. During the summer of 1923 the samples were taken about twice each month, but much of the work had to be abandoned during the autumn and winter. In 1924 it was possible to obtain samples during a longer period, but the sampling was done less frequently. In 1925 samples were collected in every month except January.

The samples were taken from various locations in the fields and in summer were selected at varying distances from the beets, some being taken with many small beet roots in them, while others were collected at considerable distance from the beets.

The temperature of the soil at each 2-inch depth was taken by pushing a rod into the side of the pit made when the tube was dug out. (Tables 1 and 2.[1]) The rod was then pulled out and a thermometer inserted in the hole.

The moisture content of the soil at the various depths was determined by taking 200 grams from each 2-inch sample of soil and drying it 24 hours at 103° C. (Tables 3 and 4.)

TABLE 1.—*Soil temperatures, field No. 1*

1923

Depth (inches)	May 16	May 22	June 7	June 16	June 23	July 17	July 25	Aug. 1	Aug. 13
	°C.	°C	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1–2	19.0	14.0	28 0	21.0	24.5	27.5	24.5	24.0	26.2
3–4	17.0	13.0	22.0	19.0	22.0	23.0	23.8	23.2	24.1
5–6	16.0	12 0	20.0	17.5	19.5	21.5	23.0	22.3	22.0
7–8	15.0	12.0	18.0	17.0	18.2	21.2	23.0	21.5	21.7
9–10	14.0	11.5	17.0	16.5	17.0	21.0	22.5	20.5	20.9
11–12	13.0	11.5	16.5	16.0	16.0	21.0	22.0	20.0	20.2
13–14	12.5	11.0	15.2	16.0	16.0	20.0	22.0	19.5	20.0
15–16	12.1	10.5	15.0	16.0	15.0	20.0	22.0	19.2	19.9
17–18	11.5	10.0	15.0	16.0	15.0	20.5	21.3	19.1	19.8
19–20	10.5	10.0	14.5	15.8	14.8	20.0	21.1	19.0	19.5
21–22	10.0	9.5	14.5	15.5	14.8	20.0	20.7	19.0	19.2
23–24	10 0	9.0	14.5	15.0	14.5	19.5	20.5	18.8	19.2

1925

Depth (inches)	Feb. 6	Mar. 13	Apr. 18	May 23	June 10	June 29	July 17	Aug. 3	Aug. 22	Sept. 2	Oct. 17	Nov. 21	Dec. 26
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1–2	0.0	1.0	15.0	27.0	19 0	24.0	25.0	25.0	19.0	20.0	6.0	1.0	0.5
3–4	0.0	1.0	13.0	25.0	17.0	23.0	25.0	23.0	18.5	19.5	5.0	1.0	0.8
5–6	0.7	1.2	11.5	21.5	15.0	23.0	25.0	22.5	18.5	19.0	5.0	1.0	1.0
7–8	1.0	1.3	11.0	19.0	15.0	22.7	25.5	22.5	19.0	18.5	5.5	1.5	1.2
9–10	1.0	2.0	10.5	18.5	15.0	22.7	26.0	22.0	19.5	18.5	6.0	2.0	1.5
11–12	1.0	2.2	10.0	18.0	15.0	22.5	26.5	22.0	20.0	18.5	7.0	2.5	2.2
13–14	1.5	2.2	10.0	17.0	15.0	22.0	26.0	22.0	20.0	18.5	8.0	2.7	2.4
15–16	1.5	2.4	9.5	17.0	15.0	22.0	26.0	22.0	20.0	19.0	8.5	3.0	2.6
17–18	1.8	2.5	9.5	16.0	15.0	21.0	25.5	22.0	20.0	19.0	9.0	3.3	2.8

[1] To save space the tables for 1924 are omitted here but are represented on the charts shown on p. 284.

TABLE 2.—*Soil temperatures, field No. 2*

1923

Depth (inches)	May 16	May 31	June 11	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29	Sept. 5	Nov. 6
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1–2	12.0	13.7	29.0	30.0	28.0	34.0	35.0	30.0	30.0	30.0	12.5
3–4	13.0	13.5	26.0	26.0	25.0	30.0	31.5	28.0	27.0	28.5	9.5
5–6	13.0	13.0	24.0	24.0	24.0	28.0	29.0	25.5	26.5	26.5	7.5
7–8	12.5	13.0	23.0	22.0	22.0	26.0	28.0	24.0	25.8	25.2	6.5
9–10	12.5	13.0	22.0	22.0	21.0	25.0	27.5	23.0	24.5	23.5	6.25
11–12	12.5	13.0	22.0	22.0	21.0	24.0	26.5	23.0	24.0	23.5	6.25
13–14	12.5	13.5	22.0	21.5	21.0	23.0	26.0	22.5	24.0	22.5	7.0
15–16	12.5	14.0	22.0	21.0	21.0	22.0	25.5	22.5	23.8	21.5	7.2
17–18	12.5	14.0	22.0	21.0	21.0	22.5	25.0	21.8	23.8	22.2	8.0
19–20	12.5	14.0	23.0	21.5	22.0	23.0	25.0	21.5	23.5	20.0	8.0
21–22	12.5	14.0	24.0	22.0	22.5	23.0	25.0	22.0	23.5	20.0	8.5
23–24	12.5	14.0	24.0	22.5	23.0	23.0	25.5	22.0	23.5	19.8	9.0

1925

Depth (inches)	Feb. 26	Mar. 20	Apr. 9	May 13	June 24	July 9	Aug. 6	Sept. 10	Oct. 24	Nov. 27	Dec. 18
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1–2	15.0	17.0	19.0	19.5	24.0	29.0	28.0	15.0	11.0	5.5	0.0
3–4	14.5	15.0	16.0	19.0	22.5	27.0	26.0	14.5	10.5	4.0	0.0
5–6	13.5	11.0	14.0	18.0	22.25	24.0	25.0	14.5	9.5	3.5	0.3
7–8	13.0	9.0	12.0	18.0	22.0	23.0	24.0	14.5	9.0	3.0	1.0
9–10	13.0	8.0	11.0	17.5	22.0	22.0	24.0	15.0	9.0	3.0	1.2
11–12	13.0	7.5	10.0	17.0	22.0	22.0	23.0	16.0	9.0	3.2	1.5
13–14	13.2	7.0	10.0	17.0	22.0	22.0	23.0	16.0	9.5	3.5	1.5
15–16	13.5	6.5	10.0	16.5	22.0	22.0	23.0	17.0	9.6	3.7	2.0
17–18	14.0	6.0	10.0	16.5	22.0	22.0	23.0	17.0	10.0	4.0	2.3

TABLE 3.—*Soil moisture, field No. 1*

1923

Depth (inches)	May 16	May 22	June 7	June 16	June 23	July 17	July 25	Aug. 1	Aug. 13
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1–2	12.0	8.5	10.0	4.0	10.0	4.0	8.0	8.0	2.0
3–4	13.5	13.5	14.0	9.0	12.0	5.5	5.0	8.0	4.0
5–6	14.0	12.5	14.0	11.0	12.0	6.0	5.0	5.5	4.5
7–8	14.0	15.0	16.0	12.0	12.0	6.0	5.5	4.5	5.0
9–10	14.0	15.0	14.5	12.5	14.0	6.5	5.5	5.5	8.0
11–12	13.5	15.0	15.5	13.5	13.5	5.5	5.0	4.5	8.5
13–14	14.5	15.0	15.0	13.0	12.0	5.5	5.0	5.0	9.0
15–16	15.0	20.0	17.0	14.5	13.0	7.0	5.0	6.0	10.0
17–18	17.0	20.0	16.0	14.0	13.0	7.0	5.0	7.0	10.5
19–20	17.0	23.5	14.5	15.0	13.5	7.0	5.5	7.5	11.5
21–22	17.0	23.5	15.5	15.5	15.0	9.0	6.5	8.5	12.5
23–24	16.5	22.5	15.0	15.0	15.0	9.0	7.5	10.5	14.0

1925

Depth (inches)	Feb. 6	Mar. 13	Apr. 18	May 23	June 10	June 29	July 17	Aug. 3	Sept. 2	Oct. 17	Nov 21	Dec. 26
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
1–2	21.0	21.0	15.0	9.5	10.5	6.5	9.5	4.5	8.5	8.0	12.0	14.5
3–4	21.0	18.0	16.0	14.5	13.0	12.0	10.0	9.0	11.5	10.5	11.5	13.5
5–6	21.0	19.0	16.5	14.0	15.0	13.0	11.0	10.5	11.5	11.5	12.0	13.5
7–8	20.5	17.0	16.0	14.0	15.0	13.0	11.5	10.5	12.0	12.5	12.0	13.0
9–10	18.0	17.5	16.0	15.0	15.5	14.5	11.5	11.5	12.5	12.0	11.0	12.5
11–12	18.5	18.0	17.0	14.5	17.5	13.5	12.0	11.0	13.5	12.5	10.5	12.5
13–14	19.0	19.5	18.0	17.0	16.5	14.5	15.5	11.5	14.0	12.5	10.5	12.5
15–16	18.0	20.0	18.0	17.5	17.5	15.5	15.0	13.0	15.5	11.5	11.5	13.0
17–18	17.5	18.5	17.5	16.0	17.5	15.5	14.5	12.5	15.5	11.5	11.5	13.0

TABLE 4.—*Soil moisture, field No. 2*

1923

Depth (inches)	May 16	May 31	June 11	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29	Sept. 5	Nov. 6
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1-2	10.5	10.5	10.0	10.5	9.5	7.5	5.5	4.5	3.5	9.0	10.5
3-4	14.5	14.0	12.0	13.0	14.0	11.0	11.5	10.5	7.5	11.0	11.0
5-6	15.0	13.5	11.5	15.0	14.5	11.0	11.5	10.5	8.5	11.0	12.0
7-8	16.0	14.5	12.0	14.5	14.0	11.0	11.5	10.5	8.0	11.5	12.0
9-10	14.5	13.5	13.0	15.0	13.0	11.5	11.5	11.0	8.5	11.5	12.5
11-12	15.5	13.0	12.5	15.0	13.0	11.0	11.0	10.5	8.5	11.5	12.0
13-14	15.0	12.0	12.5	14.5	13.0	11.0	10.5	10.0	8.0	12.5	11.5
15-16	14.5	13.0	12.0	13.5	13.0	12.0	11.0	10.0	8.5	12.5	12.0
17-18	15.0	13.5	11.0	13.5	12.5	11.5	11.0	11.0	8.5	13.0	11.5
19-20	15.0	13.5	11.0	12.0	13.0	11.5	11.0	11.5	9.0	12.5	11.5
21-22	15.0	14.0	11.0	12.5	12.5	11.5	11.0	11.5	9.5	12.5	11.0
23-24	14.5	18.5	11.0	11.5	12.5	11.5	11.5	11.5	9.5	12.0	11.0

1925

Depth (inches)	Feb. 26	Mar. 20	Apr. 9	May 13	June 24	July 9	Aug. 6	Sept. 10	Oct. 24	Nov. 27	Dec. 18
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1-2	14.0	9.5	8.5	5.0	5.5	12.0	6.5	11.0	10.0	17.0	15.5
3-4	15.0	10.0	11.0	9.0	10.0	13.0	8.5	11.0	12.5	18.5	16.0
5-6	16.5	14.0	13.0	11.5	12.0	13.5	10.0	12.0	12.5	19.0	15.5
7-8	18.0	16.0	14.5	12.5	13.0	14.5	10.5	11.5	12.0	19.0	15.5
9-10	16.0	17.5	14.0	12.0	13.5	14.5	11.0	12.5	12.5	18.5	14.0
11-12	14.0	14.0	13.5	11.0	12.0	13.5	10.5	12.5	11.5	18.5	14.0
13-14	17.0	13.0	13.0	11.0	11.0	13.5	11.0	13.5	10.5	18.0	15.0
15-16	13.0	14.0	13.0	10.5	11.0	13.0	10.5	13.5	11.0	18.0	15.0
17-18	13.5	13.0	12.5	10.0	11.5	13.0	11.0	13.5	10.5	17.5	15.0

During the summer of 1923 a detailed study was made of all nema species in these fields (Table 5) and the numbers and depths at which they occurred. Observations were made on the life history by noting the stages of growth of the individuals and the times at which the females were found producing eggs.

In 1924 and 1925 this study was limited to *Heterodera schachtii*, the mononchs, and a few species found inhabiting the cysts of *H. schachtii*. It was necessary thus to limit the work because of the great amount of time necessary for making identifications and counts of the individuals found. From observations during the last two years of the experiment and occasional counts, it was found that the data for 1923 were in a large measure typical of those for other years. The species occurring in greatest numbers were usually found in most of the samples, while the more rare forms sometimes occurred in a few samples. Occasionally a single specimen or a small number of a certain species appeared in a single sample and no others were ever found.

Samples in which Rhabditis and some of the Dorylaimus were very numerous were subdivided and an estimate made, based on the count of a small portion. This was never done for mononchs and other less numerous species.

The number of mononchs found and the depths at which they occurred are shown in detail in Tables 6 to 9, inclusive.

TABLE 5.—*Number and species of nemas in field No. 1* *ᵃ and in field No. 2 ᵇ, 1923*

Species	Field No. 1	Field No. 2
Heterodera schachtii (adults, larvae, and eggs)	101,331	103,602
Rhabditis (mostly larvae of several species)	2,059	5,684
Dorylaimus obtusicaudatus	1,340	1,364
Nygolaimus sp	884	704
Mononchus macrostoma	823	3
Mononchus parabrachyurus	752	
Trichodorus obtusus	505	
Acrobeles butschlii	179	552
Achromadora minima	145	
Cylindrolaimus obtusus	110	31
Tylenchus agricola and T. filiformis	66	262
Tylenchus sp		546
Prismatolaimus stenurus	56	9
Aphelenchus modestus	23	320
Aphelenchus agricola	22	
Dorylaimus regius	18	74
Microlaimus sp	12	
Monhystra sp	12	14
Dorylaimus carteri	10	52
Dorylaimus monhystra	9	
Cephalobus sp	9	
Acrobeles complexus	7	70
Bastiania exilis	6	
Cephalobus nanus	5	
Tylenchus clavicaudatus	4	
Cephalobus subelongatus	3	201
Cephalobus oxyuroides	3	384
Discolaimus texanus	1	
Plectus parietimus	1	26
Plectus sp		58
Mononchus sigmaturus		1,152
Mononchus papillatus		447
Agamermis sp		161
Acrobeles symmetricus		78
Aplectana sp		35
Xiphinema americana		17
Alaimus sp		10
Discolaimus sp		3
Total	108,395	115,859
Mononchs, number of individuals	1,575	1,602
Other free-living species, number of individuals	5,491	10,655
Mononchs, percentage of free-living individuals	22.2	13.1
Heterodera schachtii, per acre population	11,259,000,000	14,800,000,000
Mononchs, per acre population	175,000,000	145,636,000
Other species, per acre population	610,111,000	968,636,000
Total nema population per acre	12,044,111,000	15,914,272,000

ᵃ On the Bullen farm, near Lewiston, Utah. ᵇ On the Davis farm, near Salem, Utah.

TABLE 6.—*Number of Mononchus macrostoma, field No. 1*

1923

Depth (inches)	May 16	May 22	June 7	June 16	June 23	July 17	July 25	Aug. 1	Aug. 13
1-2		24	2	7		1	4		2
3-4		47	6	73	3		51	3	7
5-6		8	30	128	3	6	87	5	80
7-8		4	20	29	2	6	11	33	29
9-10			3	30	2	4	7	29	1
11-12			2				4	2	2
13-14							1		
15-16	5			2					
17-18	6							1	
19-20							1		
21-22			3				1	1	
23-24								3	
Total	11	83	66	269	10	17	167	77	121

Grand total, 821; population per acre, 91,444,000.

In 1924 only three specimens of *Mononchus macrostoma* were found in field No. 1. Two of these were found October 30, and the other one November 28. On this basis the nema population per acre would be 333,000.

The following numbers of *Mononchus macrostoma* were found in field No. 1 in 1925:

Date	Number	Date	Number
Feb. 6	0	Aug. 3	3
Mar. 13	2	Sept. 2	2
Apr. 18	9	Oct. 17	0
May 23	0	Nov. 21	0
June 10	0	Dec. 26	0
June 29	1		
July 17	1	Total	18

Population per acre, 1,500,000.

TABLE 7.—*Number of Mononchus parabrachyurus, field No. 1*

1923

Depth (inches)	May 16	May 22	June 7	June 16	June 23	July 17	July 25	Aug. 1	Aug. 13
1–2	7	18							
3–4	4	19	2	13	13		4		8
5–6	14	3		20	2	3	14	9	36
7–8	8	2	7	12	9	22	8	39	31
9–10	4		23	11	18	68	31	77	24
11–12			5	1	22	21	8	31	22
13–14			2	2			1	5	5
15–16			4	1				2	16
17–18			4						
19–20			3					1	5
21–22					1			3	2
23–24								1	1
Total	37	42	50	60	65	114	66	168	150

Grand total, 1923, 752; population per acre, 83,555,000.

1924

Depth (inches)	May 22	June 10	June 24	July 7	July 20	Aug. 18	Sept. 24	Oct. 30	Nov. 28
1–2									1
3–4	8	4		2		2	5	1	41
5–6	21		9	3	8	8	22	4	12
7–8	14		13	50	11	16	15	14	12
9–10	3		4	78	49	14	3		24
11–12	1			13	7	3	2	5	7
13–14	2	1	2	7	4				1
15–16	1		3	8					
17–18	1			3					
Total	51	5	31	164	79	43	47	24	98

Grand total, 1924, 542; population per acre, 72,888,000.

TABLE 7.—*Number of Mononchus parabrachyurus, field No. 1*—Continued

1925

Depth (inches)	Feb. 6	Mar. 13	Apr. 18	May 23	June 10	June 29	July 17	Aug. 3	Sept. 2	Oct. 17	Nov. 21	Dec. 26
1-2			1								5	5
3-4	2		15	1			2	1	5	18	6	12
5-6	11		12	5	4	2	2	4	6	9	1	2
7-8	6		5	4	11	4	7	5	4	25		1
9-10	1			7	3	4	5	3	1	24		3
11-12				1	1	2	2	2	1	13		3
13-14						2	2		1	3		
15-16						1	1		2			
17-18												
Total	20		33	18	19	15	21	15	20	92	12	26

Grand total, 1925, 291; population per acre, 24,250,000.

TABLE 8.—*Number of Mononchus sigmaturus, field No. 2*

1923

Depth (inches)	May 16	May 31	June 11	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29	Sept. 5	Nov. 6
1-2		1			2						5
3-4	9	42	19	6	13		41	3	4	4	7
5-6	22	70	21	17	28	7	99	4	10	18	26
7-8		71	27	15	64	3	39	30	10	26	30
9-10	20	107	13	7	19	3	58	8		41	6
11-12		6	4			1	1	5		7	1
13-14	2	24					1			14	
15-16											
17-18		1	1				1				
19-20		3								3	
21-22		2									
23-24											
Total	53	327	85	45	126	14	240	50	24	113	75

Grand total, 1,152; population per acre, 104,727,000.

1924

Depth (inches)	Apr. 6	May 20	June 2	June 17	June 30	July 15	July 28	Aug. 12	Aug. 25	Oct. 3	Nov. 21
1-2											
3-4		3			4	2	4	6			3
5-6	5		1		1	8		8	6	3	1
7-8		2	3		17	4	8	3	5	6	1
9-10	1	2	3			3	4	3	4	2	3
11-12				7		3		3			
13-14				3		2	1	2			
15-16				1			3				
17-18							3				
Total	6	7	7	11	22	22	23	25	15	11	8

Grand total, 157; population per acre, 14,272,000.

TABLE 8.—*Number of Mononchus sigmaturus, field No. 2*—Continued

1925

Depth (inches)	Feb. 26	Mar. 20	Apr. 9	May 13	June 24	July 9	Aug. 6	Sept. 10	Oct. 24	Nov. 27	Dec. 18
1–2											17
3–4	10		1		4	1			2	6	1
5–6	1		5		3	3	2		6	9	5
7–8				6	4	3	3	1	3	9	1
9–10	1	1	1		5	2	1		3	5	24
11–12		4			2	2	1				5
13–14											
15–16											
17–18					1						
Total	12	5	7	6	19	11	7	1	14	29	53

Grand total, 164; population per acre, 14,090,000.

TABLE 9.—*Number of Mononchus papillatus, field No. 2*

1923

Depth (inches)	May 16	May 31	June 11	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29	Sept. 5	Nov. 6
1–2											1
3–4		1	5		2	1	19				4
5–6	1	2		3	22		28			2	
7–8	16	3	4	6	47	4	5				2
9–10	23	12		8	11	1	4			6	
11–12		4	3	8	3	1		6		2	
13–14	45	7	4		5	3		3		13	2
15–16	29	3	5	1	4			1		3	2
17–18	11	3	1		2					6	
19–20	5	4	2		1					4	
21–22	1	2	1								
23–24		2	1							1	
Total	131	43	26	26	97	10	56	10		37	11

Grand total, 447; population per acre, 40,636,000.

In 1924 the following numbers of *Mononchus papillatus* were found in field No. 2:

Date	Number	Date	Number
April 6	1	August 12	0
May 20	6	August 25	1
June 2	3	October 3	0
June 17	0	November 21	1
June 30	0		
July 15	3	Total	15
July 28	0		

Population per acre, 363,000.

Of the 11 soil samples taken from field No. 2 between February 26, and December 18, 1925, only four contained mononchs. Those taken March 20, June 24, July 9, and November 27 contained one each. The population per acre was 363,000.

No *Mononchus parabrachyurus* individuals were found in field No. 2 in 1923.

In field No. 2 the following numbers of *Mononchus parabrachyurus* were found in 1924:

Date	Number	Date	Number
April 6	0	August 12	3
May 20	0	August 25	8
June 2	2	October 3	3
June 17	3	November 21	3
June 30	14		
July 15	4	Total	46
July 28	6		

Population per acre 4,181,000.

In 1925 the distribution of *Mononchus parabrachyurus* in field No. 2 was as follows:

Date	Number	Date	Number
February 26	26	September 10	0
March 20	2	October 24	3
April 9	8	November 27	6
May 23	4	December 18	2
June 24	2		
July 9	3	Total	58
August 9	2		

Population per acre, 5,272,000.

Counting the *Heterodera schachtii* was a complicated process. The larvae and males found free in the soil were counted separately. The numbers of females on the beet roots and those which had fallen off during the washing process were noted. It was also necessary to make an estimate of the eggs and larvae remaining within the brown cysts. A careful count of the eggs in 500 cysts showed that the average number in each was 280, and upon this basis the cyst contents were estimated. During the late autumn and winter months practically all of the cysts containing eggs were full, but with the coming of spring the larvae began to emerge, and from then on during the summer the cysts usually were found partially emptied, except those newly formed on the beet roots. In estimating the contents, the cysts were classed as full, 90 per cent full, 75 per cent full, 50 per cent full, 25 per cent full, 10 per cent full, and empty. When a cyst contained only a few eggs and larvae that could be counted easily, the exact number was taken. There were great numbers of empty cysts, accumulations from former years, which had to be opened in order to count the few eggs or larvae which occasionally remained. Cysts containing nothing but dead eggs or larvae were classed as empty. Most of the cysts from which a portion, or all, of the larvae had emerged were found floating on the surface of the screen residues, while the full ones were collected in the heavier screenings.

The *Heterodera schachtii* population per acre of field No. 1 was 11,250,000,000 in 1923, 2,251,000,000 in 1924, and 34,821,000,000 in 1925. Data for field No. 1 were incomplete. The *H. schachtii* population per acre of field No. 2 is shown in Table 10.

TABLE 10.—*Number of Heterodera schachtii, field No. 2*

1923 [a]

Depth (inches)	June 23	June 30	July 12	July 20	Aug. 8	Sept. 5	Nov. 6
1-2	490	1,260	31,500	610	735	212	162
3-4	280	1,148	10,366	5,611	3,154	1,114	----
5-6	140	100	10,145	3,395	436	1,000	408
7-8	----	----	6,143	486	1,447	249	375
9-10	----	3,696	2,957	1,536	2,153	411	154
11-12	----	2,156	1,795	735	1,431	386	220
13-14	----	210	----	----	611	299	470
15-16	----	----	----	----	----	1,172	986
17-18	----	----	----	201	----	150	184
19-20	----	70	----	----	----	287	168
21-22	----	----	----	----	----	100	94
23-24	----	----	----	----	----	----	----
Total	[b] 910	8,640	62,906	12,574	9,967	5,380	3,221

Grand total, 103,598; population, per acre, 14,800,000,000.

1924

Depth (inches)	June 2	June 17	June 30	July 15	July 28	Aug. 12	Aug. 25	Oct. 10	Nov. 25
1-2	288	53	68	241	377	920	908	26	109
3-4	98	56	1,443	1,355	180	1,845	3,041	98	1,231
5-6	462	252	1,414	837	413	675	2,853	420	2,457
7-8	116	71	2,472	598	849	1,437	1,470	127	1,649
9-10	694	241	1,093	1,879	186	810	841	81	3,466
11-12	----	75	1,360	210	198	398	517	8	1,701
13-14	----	14	28	88	664	223	300	81	6,003
15-16	----	32	15	75	1,590	326	945	----	30
17-18	----	21	----	----	1,278	----	1,071	----	383
Total	1,658	815	7,893	5,283	5,735	6,634	11,946	841	17,029

Grand total, 57,834; population per acre, 6,436,000,000.

1925

Depth	Feb. 26	Mar. 20	Apr. 9	May 13	June 15	June 24	July 9	July 21	Aug. 6	Aug. 15
1-2	4,589	3,312	----	----	410	2,313	2,811	----	2,544	1,809
3-4	624	1,554	500	----	1,500	2,438	3,811	65	2,024	1,767
5-6	530	2,604	175	49	1,175	693	2,704	1,260	2,935	230
7-8	298	328	32	423	1,609	1,191	1,396	3,486	1,687	28
9-10	362	1,278	2,000	62	398	668	1,577	724	4,904	275
11-12	5,471	1,245	3,486	847	944	578	2,430	66	4,303	637
13-14	949	20	----	826	4,838	1,723	3,445	1,423	5,653	2,625
15-16	6,698	68	108	290	3,683	175	710	----	772	1,427
17-18	8,123	250	175	----	598	----	352	25	350	-1,860
Total	27,644	10,659	6,476	2,497	15,155	9,779	19,236	7,049	25,172	10,658

Grand total, 134,325; population per acre, 13,444,000,000 .

[a] No record was available of the samples taken May 16, May 31, June 11, and Aug. 29.
[b] Incomplete.

In 1924 the populations of *Heterodera schachtii* of both fields were much lower than in 1923 or 1925. There are two reasons for this: (1) The year 1923 was, in general, a normal year for successful sugar-beet production, and many of the infested beets made a good growth. This meant an abundance of roots, upon which developed large populations of *H. schachtii*. However, 1924 was an exceedingly poor year for sugar-beet growth and the young beets found difficulty in becoming established. (2) The large numbers of *H. schachtii* hatching in the spring of 1924 attacked these weak seedlings

and killed practically all of those in the infested areas. This resulted in the destruction of a large percentage of the *H. schachtii* which had hatched and entered the beet roots, and only comparatively small numbers survived on the few beets remaining.

The growing season of 1925 was excellent, and the young beets were well established early in the spring. Even on the most severely infested areas many of them made a fair growth because the *Heterodera schachtii* populations were low, as the previous year had been an unfavorable one. Also the fertility of the soil was higher because on the infested areas few beets had been produced in 1924 and the soil had been kept fallow by cultivation. For these reasons, the beets grew thriftly, developing large root systems upon which the *H. schachtii* increased to such enormous numbers that by the end of 1925 the populations had again reached a high point.

It may safely be predicted that these high populations will destroy practically all of the young beets in 1926 and that by the end of the year the populations will be much lower than at the beginning.

No attempt has been made to place the *Heterodera schachtii* populations on the graphs (figs. 3 and 4) because the bulk of the population occurs in the cysts and these are moved about in the soil by plowing, cultivating, and other cultural operations until their locations have no relation to the actual point of habitat.

LIFE HISTORY OF MONONCHS IN THE FIELD

Definite broods or cycles of mononchs were not observed in any of the species studied. Every collection contained individuals of various sizes, and examination of the denticles in the pharynx showed that the smaller forms had yet to molt one or more times. Cobb[4] states that mononchs probably molt four times, and all of the observations made on the specimens collected in this experiment bear out this statement, although like Steiner and Heinly[5], the writer did not observe specimens making a first molt just after emerging from the eggs. No method was devised for learning how long the eggs lie in the soil before the larvae hatch or the rate of growth of the young mononchs. From the fact that very small individuals were found three months after the last ova-bearing females were seen, it would appear that either some of the eggs do not hatch immediately or that growth is very slow.

Temperature is apparently an important factor in egg production. In field No. 1 no gravid females of *Mononchus parabrachyurus* were found in the winter of 1924–25 during the months of October, November, February, and March. In field No. 2 gravid females were collected in October and February but none in November and March. Tables 3 and 4 show that the temperatures of both fields fell to about 10° C. or lower on the dates when gravid females were not found.

In field No. 2, *Mononchus sigmaturus* were found gravid on November 27, 1925, when the temperature was below 5° C. In January, 1926, they were found gravid when the temperature was 2.5°. This

[4] COBB, N. A. THE MONONCHS. (MONONCHUS BASTIAN 1866.) A GENUS OF FREE-LIVING PREDATORY NEMATODES. Soil Sci. 3: 431–486, illus. 1917.
[5] STEINER, G., and HEINLY, H. THE POSSIBILITY OF CONTROL OF HETERODERA RADICICOLA AND OTHER PLANT-INJURIOUS NEMAS BY MEANS OF PREDATORY NEMAS, ESPECIALLY BY MONONCHUS PAPILLATUS BASTIAN. Jour. Wash. Acad. Sci. 12: 367–386. illus. 1922.

indicates that they reproduce at much lower temperature than do *M. parabrachyurus.* No gravid females were found on December 18, 1925, when the soil was frozen to a depth of 8 inches. After a box of the soil had remained in the laboratory at ordinary room temperatures until December 23, gravid females were numerous. This would indicate that there is prompt response to a rise in temperature at that time of year.

On August 8, 1923, about 50 pounds of soil from field No. 2 was placed in a cool cellar. At that time a count showed 12 *Mononchus sigmaturus* per pound. On December 6, they had increased to 308 per pound, but on March 8, 1924, only a few were to be found. Nothing was determined as to the cause of their disappearance.

Cessation of egg production during the summer months is perhaps an adaptation to the arid conditions which prevailed in these soils before the advent of irrigation, but is more probably caused by the high temperature of the soil at this time. That such is the case seems to be borne out by the fact that these same species of mononchs collected from the cool, moist soils along canyon and valley streams often are found producing eggs during the summer months. This may be due to the absence there of the high temperatures common in the valley fields or perhaps to the development of biologically adapted strains of the same species. No transfers of mononch colonies have been made to determine this point.

No gravid females were found among the 823 specimens of *Mononchus macrostoma* collected in 1923. Many were adults, but it was evident that in this field the period from May 16 to August 13 was not the time of egg production. Specimens collected in other fields during the months from November to March contained eggs, except those collected during very cold periods. Immature specimens are almost always present in collections made at any time of year. Specimens from stream banks and springs often are gravid during the summer months.

Considerable numbers of gravid females of *Mononchus parabrachyurus* were found in October, and occasional specimens occurred during the winter months when the temperature was above 10° C. However, the greater number appeared in the April and May collections, and this is probably the time of maximum egg production.

Gravid females of *Mononchus papillatus* were numerous in the April and May collections, but none were found later. Immature specimens were numerous during the summer months, again pointing to a delayed hatching or slow growth of the larvae. Two gravid specimens were found in September, 1923, but none in November.

In rare instances gravid females of *Mononchus sigmaturus* have been collected in July and August, but the greatest numbers appeared in the March, April, and May collections. Egg production apparently ceases at about 2.5° C.

CAUSE OF REDUCTION IN MONONCH POPULATION

The most striking fact shown by the data on mononch population (Tables 6 to 9, and text lists on p. 270) is the reduction in the numbers of all species, with the one exception of *Mononchus parabrachyurus* in field No. 2, where this species was first found in 1924.

Mononchus macrostoma, so abundant in field No. 1 in 1923, apparently was just as numerous in 1922 when the colony was discovered. Between August 13, 1923, and May 22, 1924, they practically disappeared from the field, as is shown in Table 3. The cause is not definitely known, but in July and August, 1923, several specimens were found that were heavily parasitized by elongate-ellipsoidal sporozoans. In some instances the body cavities were almost completely filled, especially about the region of the reproductive organs. Unfortunately, these specimens were lost and a definite description of the parasites can not be given. Similar parasitized specimens have not been collected from other localities.

Specimens of *Mononchus parabrachyurus* have frequently been found parasitized by sporozoans (fig. 1). In rare instances sporozoans apparently cause the death of the mononch. The sporozoans usually congregate in large numbers about the reproductive organs (fig. 1), and such infested specimens have not been found producing eggs. In February, 1926, 30 per cent of the mononchs in field No. 1 were parasitized, and there was little doubt that these sporozoans were the cause of the reduction in numbers. A few specimens contained rodlike bacteria in addition to the sporozoans (fig. 2).

Sometime after specimens had been collected from field No. 1 about 200 specimens were collected from fields near it, but in none of them were parasitized specimens found. The attack was therefore confined to that one field and was not epidemic to the whole section.

Mononchus sigmaturus has also shown a steady reduction in numbers, but no parasites ever have been observed in any of the specimens examined from field No. 2 or other neighboring fields. Dead specimens frequently have been found, but nothing could be seen in the bodies that would indicate the cause of death. A specimen collected at Ogden, Utah, was heavily infested with a sporozoan similar to, perhaps identical, with one found in *M. parabrachyurus.*

Mononchus papillatus almost disappeared from field No. 2 during the winter of 1923–24, but nothing is known of the cause. This species is less frequently found inhabiting cultivated fields, and observations on it have been limited.

FOOD HABITS OF MONONCHS

Mononchus macrostoma has been observed feeding on nemas, rotifers, and other microscopic soil organisms. The bulk of its food appears to be organisms other than nemas. Of the nemas devoured, the majority were Rhabditis, and only three were *Heterodera schachtii*—two larvae and one male. Rhabditis apparently is preferred because the cuticle is thin and the body contents easily drawn out. The cuticle usually is discarded, but occasionally it is swallowed.

Mononchus parabrachyurus eats as its usual food rotifers, protozoans, and other microorganisms. Nemas rarely are devoured, the only records obtained being one Rhabditis and one *Heterodera schachtii* larvae. The cuticle of both specimens was discarded. It was impossible to get many of these mononchs to attack other nemas when they were kept constantly about them for as long as two weeks at a time. They apparently were able to obtain minute organisms from the soil particles and water in which they were kept. Sometimes they would attack other nemas, but after a brief struggle would
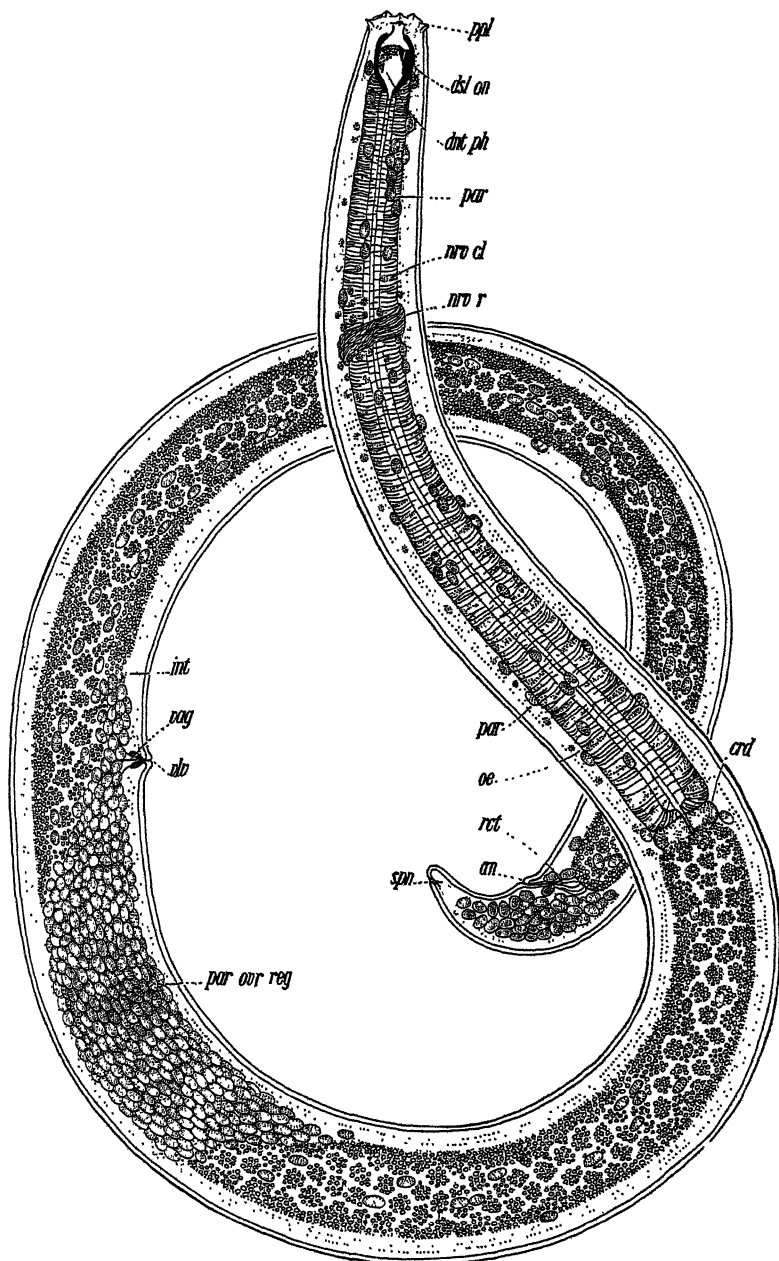
FIG. 1.—*Mononchus parabrachyurus* infested with sporozoan parasites which have reached all parts of the body, congregating especially about the region containing the reproductive organs. Such large colonies sterilize the mononch and prevent the production of eggs

Key.—an, anus; *crd*, cardia; *dnt ph*, denticles of pharynx; *dsl on*, dorsal tooth; *int*, intestine; *nrv cl*, nerve cell; *nrv r*, nerve ring; *oe*, esophagus; *par*, parasite sporozoan; *par ovr reg*, parasite in ovary region; *ppl*, papillae; *rct*, rectum; *spn*, spinneret; *vag*, vagina; *vlv*, vulva.

release them.   They appeared to be handicapped in not having firm soil particles on which to anchor themselves by the spinneret.   Under field conditions they may more successfully attack and devour nemas, but from laboratory observations it appears that they prefer other forms of prey.

Mononchus sigmaturus, like *M. parabrachyurus*, appears to prefer microorganisms other than nemas for food.   It frequently attacked the eggs of *Heterodera schachtii* when the cyst had been broken open, and sometimes it was able to puncture both the eggshell and cuticle of the larvae and draw out the body contents (fig. 5).   Small nemas were attacked occasionally but often were released without having been punctured or killed.
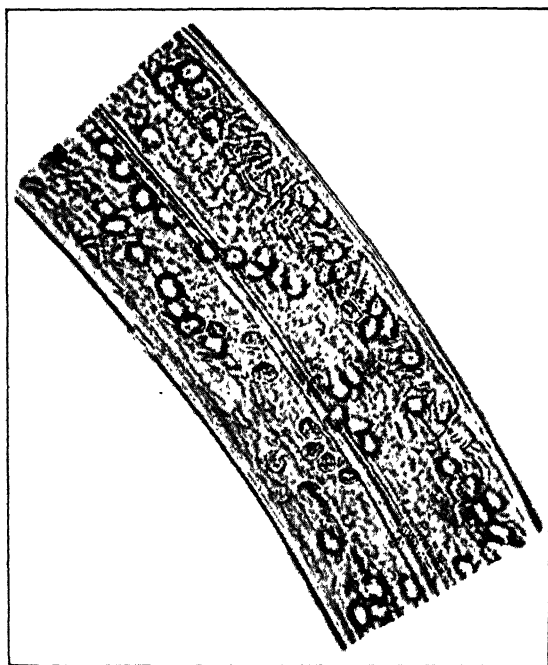
FIG. 2.—Photomicrograph of neck of *Mononchus parabrachyurus*, showing sporozoan parasites and rodlike bacteria in the body cavity

Mononchus papillatus was found by Steiner and Heinly,[6] to be especially voracious and to attack other nemas readily.   Several times during the summer of 1923, when the screen residues from the soil samples were examined, these mononchs were found devouring other nemas.   Even the large males of *Heterodera schachtii* are readily attacked and the body contents drawn out (fig. 4).   It is not unusual to find partly devoured nemas in soil where these mononchs are numerous, perhaps bearing out the findings of Steiner and Heinly that they kill for pleasure when their appetites have

[6] STEINER, G., and HEINLY, H.   THE POSSIBILITY OF CONTROL OF HETERODERA RADICICOLA AND OTHER PLANT-INJURIOUS NEMAS BY MEANS OF PREDATORY NEMAS, ESPECIALLY BY MONONCHUS PAPILLATUS BASTIAN.   Jour. Wash. Acad. Sci. 12: 367–386, illus. 1922.
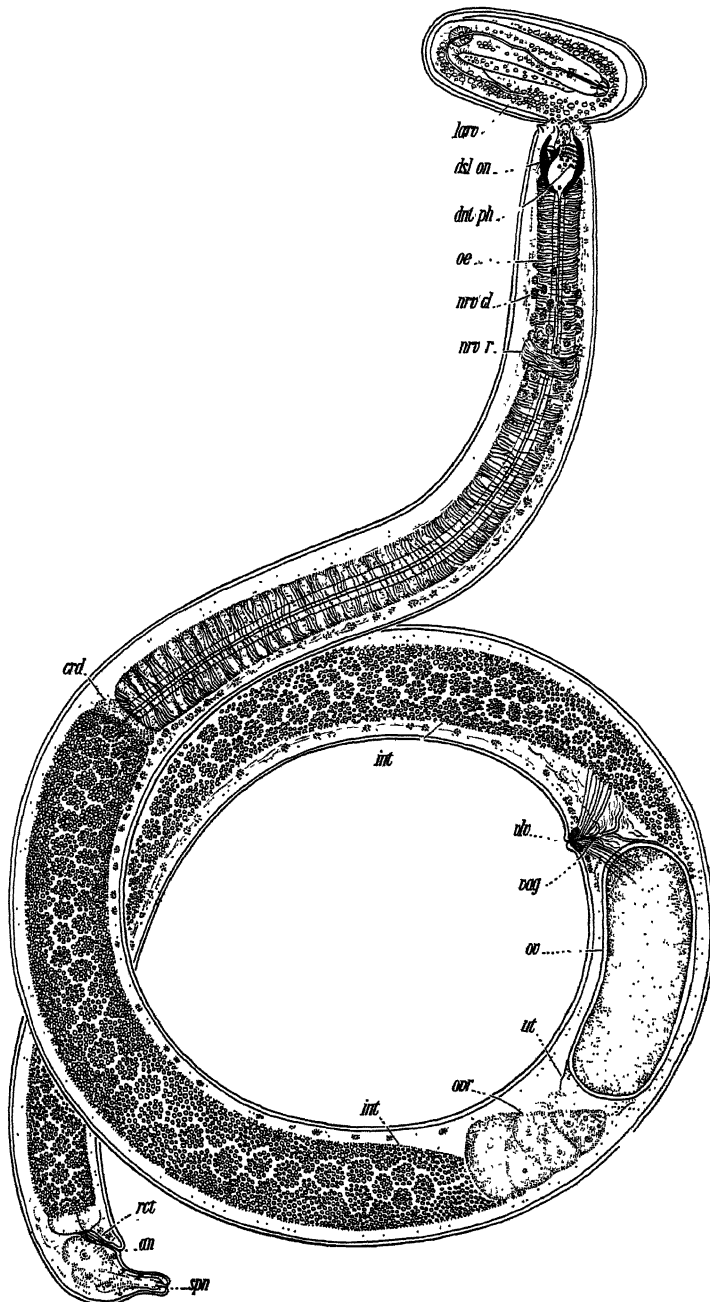
Fig. 3.—*Mononchus sigmaturus* devouring an egg of *Heterodera schachtii*. The larva (larv) can be seen coiled in the egg. The mononch has punctured both the egg and the cuticle of the larva and is drawing out the granular contents of the body

Key.—an, anus; *crd*, cardia; *dnt ph*, denticles of pharynx; *dsl on*, dorsal tooth; *int*, intestine; *larv*, larvae; *Heterodera schachtii*; *nrv cl*, nerve cell; *nrv r*, nerve ring; *oe*, esophagus; *ovr*, ovary; *ov*, ovum or egg; *rct*, rectum; *spn*, spinneret; *ut*, uterus; *vag*, vagina; *vlv*, vulva.

been satisfied. Immature females of *H. schachtii* attached to beet rootlets were fed to these mononchs, and on two occasions a mononch was observed attacking one of them and drawing out the body contents.

ECONOMIC IMPORTANCE OF MONONCHS

From the present evidence it appears that the native mononchs of the sugar-beet fields of Utah are subject to the attacks of sporozoans and other parasites or diseases that frequently destroy almost the entire colony. Under such conditions there will be no opportunity for the mononchs to become of economic importance. It might be possible, however, to transfer uninfected individuals from other localities and establish colonies of species unknown in a locality, so that they might escape the destructive parasites and diseases. But such procedure would be attended by many difficulties.[7]

The food habits of *Mononchus sigmaturus* and *M. parabrachyurus* indicate that they have but little, if any, economic importance. *M. macrostoma* is also of doubtful value.

Mononchus papillatus would doubtless be of considerable aid in the control of *Heterodera schachtii* if some method could be devised for maintaining large colonies in the infested fields.

The greatest increase in the number of mononchs occurs in April and May when other free-living forms also are exceedingly numerous. At this time there are many *Heterodera schachtii* larvae moving from the cysts to the sugar beets and some of them are destroyed by the mononchs.

Fig. 4.—Photomicrograph of head of *Mononchus papillatus* devouring a small male *Heterodera schachtii*. The body contents are being sucked out, but the cuticle is discarded

However, the mononchs seem to prefer the various species of Rhabditis, which are even more abundant than *H. schachtii* at th.s time of year. The enormous populations of *H. schachtii* that develoᴵp during the summer would not have a stimulating effect on t he mononch population because reproduction does not occur at that time.

[7] COBB, N. A. TRANSFERENCE OF NEMATODES (MONONCHS) FROM PLACE TO PLACE FOR ECONOMIC PURPOSES. Science (n. s.) 51: 640–641. 1920.

IMPORTANCE OF MOISTURE

Water is the most vital factor in nemic activity. A film about the soil particles is essential for movement and the continuation of life processes. Without it nemas must enter a quiescent form in which they lie dormant until its return.

How long this dormant condition may continue without death to the nema probably varies with the different species, but it is certain that it is normal for those here discussed to remain in this condition during the hot dry summer months. That such is the case has been determined definitely, for practically all the species here listed, by examining specimens in soil taken from unirrigated fields during the summer months.

During this quiescent stage the mouth apparently is closed by a sort of plug, the nema is inert, and respiration practically ceases. Response to moisture is almost immediate, and it is only by working rapidly that one can wash and examine a dry soil sample before the nemas begin to show signs of life. The mononchs usually revived in from 10 to 20 minutes.

It seems peculiar that some species prefer to remain in the first few inches of soil where drying out is frequent. Other species live deeper in the soil, but often some individuals of these species will be found quiescent in the hot dry soil when at a depth of 6 or 8 inches there is ample moisture, indicating that they sometimes apparently make no effort to avoid passing into this quiescent condition.

In the two fields under study nemic activity ceased when the soil-moisture content dropped to 7 or 8 per cent. This figure no doubt would vary slightly for soils of different textures. The mean moisture and temperature points for these fields are indicated in Figure 5.

The data given in Tables 6 to 9, inclusive, show that mononchs generally avoided the upper 2 inches of soil except when the moisture content was high and the temperature low. For example, in field No. 1, on May 22, 1923, both *Mononchus macrostoma* and *M. para-brachyurus* were gathered near the surface after several days of cool weather and showers.

Figure 6 shows that in field No. 1 there was a general tendency for *Mononchus parabrachyurus* and *M. macrostoma* to go deeper into the soil as the moisture decreased. However, such migration also occurred when there was an increase in temperature. As is brought out later in this paper, temperature may be the more important factor.

Field No. 2 (fig. 6) had a more erratic moisture content, owing to the fact that irrigation water was applied at various intervals. There apparently is no correspondence between the average depth of habitat of *Mononchus sigmaturus* and *M. papillatus* and the moisture content of the soil.

In Figure 6 the moisture line was obtained by charting the actual percentage of moisture at its average point. The temperature line was obtained in like manner. The habitat lines for the mononchs were obtained by the same formula and represent the average points of habitat of the various species.
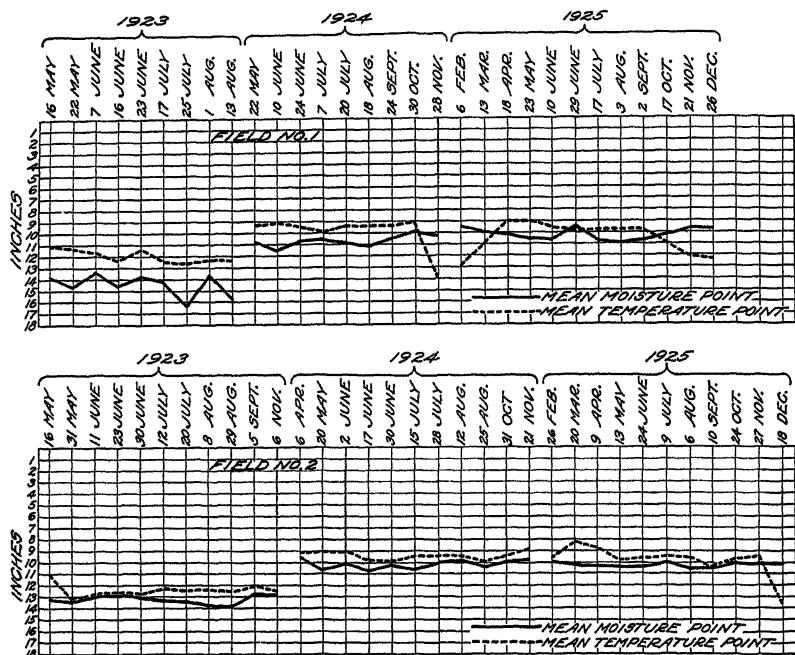
FIG. 5.—Mean moisture point and mean temperature point of fields Nos. 1 and 2, 1923, 1924, and 1925
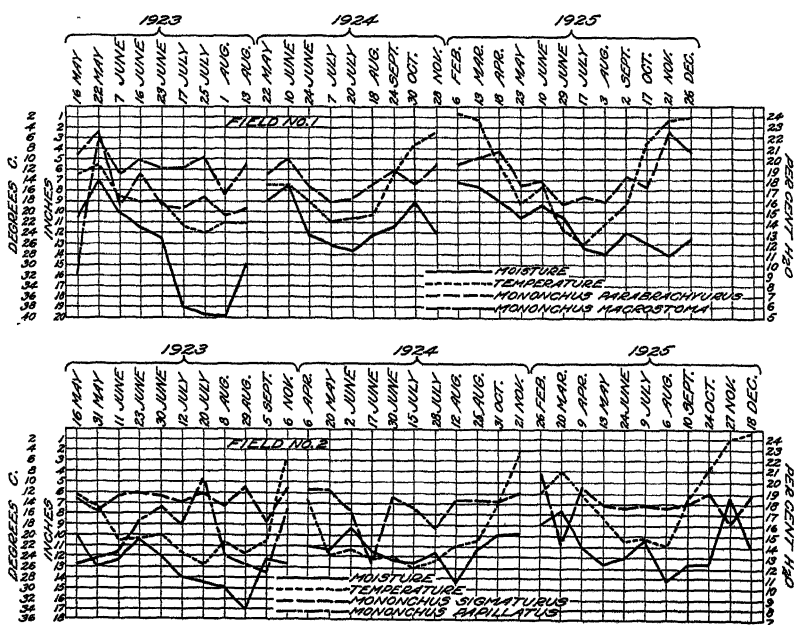
FIG. 6.—Moisture, temperature, and average points of habitat of *Mononchus parabrachyurus* and *M. macrostoma* in field No. 1, and of *M. sigmaturus* and *M. papillatus* in field No. 2, 1923, 1924, and 1925

It may be concluded from Figure 6 that mononchs are but little influenced by moisture in selecting a habitat, provided the soil contains more than the 7 or 8 per cent of moisture necessary for their activity. They do exhibit a marked tendency to avoid the upper 2 inches of soil, in which frequent drying occurs.

IMPORTANCE OF TEMPERATURE

Figure 6 shows that temperature has little influence on the point of habitat of any mononchs except *Mononchus parabrachyurus*. The occurrence of this species in field No. 1 seemed to depend largely on the temperature.

In field No. 2 the average point of habitat of *Mononchus sigmaturus* was very close to 7 inches in depth at almost all times of the year. How can the great variations in the depth at which they occurred on June 17, 1924, and March 20, 1925, be explained? Since moisture and temperature were not the cause of these variations, it seems possible that some organism preferred for food may have been present at the lower depths, causing the mononchs to migrate down to those points.

The wide variation in average point of habitat of *Mononchus macrostoma* on May 16, 1922, also seems mysterious. It seems peculiar that this species, which usually prefers the cool, moist soils of stream banks, should have lived so close to the surface in field No. 1 during the extremely hot dry summer of 1923. Observations on this species made in other fields rarely have shown that this species prefers habitats so deep as those in which they were found on May 16, 1923. Usually they are found at an average depth of from 6 to 12 inches.

The erratic average point of habitat for *Mononchus papillatus* in 1923, field No. 2 (fig. 6), seems quite typical of this exceedingly active species. It is probable from observations in this and two other fields that these mononchs migrate considerably in their search of food, without any regard whatever for temperature or moisture. However, the bulk of the specimens are almost invariably collected at a depth of 6 to 12 inches.

SUMMARY

The nemas of the genus Mononchus inhabiting the sugar-beet fields of Utah and southern Idaho are chiefly of four species: *Mononchus papillatus* Bastian, *M. macrostoma* Bastian, *M. sigmaturus* Cobb, and *M. parabrachyurus* Thorne. These species often occur in sugar-beet fields infested with the destructive sugar beet nematode, *Heterodera schachtii* Schmidt. They prefer the lighter sandy soils where they are sometimes found in populations as high as 300,000,000 per acre. In the heavy soils they occur much less frequently.

A study was made of the populations in two of these fields with the primary purpose of determining if the mononchs were of economic importance in the control of *Heterodera schachtii*. The general habits of mononchs in relation to temperature, moisture, food supply, and diseases or enemies were also studied.

The principal period of mononch reproduction occurs during the months of March, April, and May and there is probably but one generation each year. The populations vary greatly from year to year. *Mononchus macrostoma* and *M. parabrachyurus* are attacked by Sporozoan parasites that sterilize them and reduce their numbers. Other undetermined causes greatly reduced the numbers of *M. sigmaturus* and in one field practically exterminated *M. papillatus*.

Mononchus papillatus was found to be the most voracious species and frequently was observed devouring the larvae and males of *H. schachtii*. The other three species appeared to prefer rotifers and other microorganisms as food.

Because of their unstable populations, time of reproduction, and food habits it is doubtful if the mononchs studied were of economic importance in the control of the sugar-beet nematode.

Nemic activity ceased when the soil moisture content dropped to 7 or 8 per cent in the two fields under study. Mononchs are able to lie dormant through the dry summer months but revive in 10 to 20 minutes when water is applied to the soil.

Mononchus parabrachyurus appeared to migrate deeper into the soil during the hot summer months but the other species studied apparently were not influenced by temperature.

TWO WATER MOLDS CAUSING TOMATO ROOTLET INJURY [1]

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INTRODUCTION

During May, 1926, the writer isolated some possibly parasitic fungi from affected rootlets of tomato (*Lycopersicum esculentum* Mill.) seedlings in greenhouses of the United States Department of Agriculture in the District of Columbia and at the Arlington Experiment Farm, Rosslyn, Va. The underground portions of these seedlings were, on the whole, in very good condition, injury being confined usually to a relatively small portion of the more minute rootlets, which exhibited yellowish or brownish discoloration at the tip and for several millimeters to a centimeter upward.

This material while scarcely in a condition to cause practical concern, yielded nevertheless a numerous array of fungous cultures referable to more than a dozen species, most of them to a certain degree parasitic. The genus Pythium was very liberally represented in this collection, as, indeed, might be expected from the very usual association of many of its members with rootlet-tip injury in numerous representatives of the higher plants. The mycelial form identified as *Rhizoctonia solani* Kühn (*Corticium vagum* B. & C.) made its appearance in scores of cases. A less predictable feature of these isolations was a frequent recurrence of two water molds, which, because of the paucity of information as to the association of members of the Saprolegniaceae with root injury in general, deserve further discussion.

OCCURRENCE OF APHANOMYCES EUTEICHES

One of the water molds which appeared more than a dozen times was, in the absence of cross inoculation experiments, provisionally identified as *Aphanomyces euteiches* Drech., a fungus known as the cause of a very destructive type of root rot of peas prevailing especially during seasons of excessive rainfall (*4*).[2] A comparison of the strains isolated from tomato roots with others derived from pea roots showed general agreement in morphological detail, including the very distinctive peculiarity presented in the sinuous inner contour of the thick oogonial wall. Mature oospores of the fungus were recognized in the tissues of a number of affected rootlet tips. With a few exceptions the rootlets that yielded *A. euteiches* yielded no other fungus. Appearances indicated actual though not severe pathogenicity—a degree of pathogenicity important not so much on account of any potential damage to tomatoes as on account of the increased opportunity afforded the parasite for

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 3
Feb. 1, 1927
Key No. G-558

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maintaining itself until such time as an ensuing crop of peas becomes available. In regions where tomatoes and peas are cultivated on a example, in some sections of Maryland, the fungus would thus be sufficiently extensive scale to play important parts in a rotation, as for better able to survive from season to season in dangerous quantity.

PLECTOSPIRA MYRIANDRA N. G.; N. SP.

OCCURRENCE

The second water mold, *Plectospira myriandra*, was encountered about as frequently as the first, but only on potted tomato seedlings from the greenhouses at Arlington farm. Its relationship to the host appeared to be quite similar, injury being limited to brownish discoloration and eventual death of occasional rootlet tips. The damage to the material examined was rather slight. Growths originally obtained on corn-meal agar plates were freed of contaminating bacteria by the method described by Brown (*1*), which consists in making transfers from the under side of thick, plain-agar plates. Thus purified, the fungus was subsequently cultivated on artificial media like corn-meal agar, Lima-bean agar, Lima-bean decoction, and pea decoction. Advantage was also taken of the well-known method of inducing zoospore formation by the transfer of vigorous mycelia to sterile water.

MORPHOLOGY

On corn-meal agar the fungus produces a colorless growth devoid of aerial mycelium, having much the general appearance of *Aphanomyces euteiches* or *A. helicoides* Minden (*7, p. 555–562*), and exhibiting, like these, a well-defined radial effect. Under the microscope the mycelium is seen to be composed of hyphae of low refringency, branching at moderate intervals. As in the species of Aphanomyces mentioned, each hyphal element maintains approximately the same diameter from its origin to its termination. The more delicate branches, such as are produced, for example, when portions of mycelium are transferred to water (fig. 1, A) and including also the filaments bearing the antheridia, not infrequently measure less than 2μ in diameter—probably as slender mycelial elements as are known in the Saprolegniaceae.

Zoosporangia are obtained when fresh growth from liquid or agar media is transferred to water, corn-meal agar seemingly providing the most satisfactory substratum that has been used for their production. Under suitable conditions massive complexes are formed, composed of folded, inflated elements usually twice and not rarely three times the diameter of the largest vegetative hyphae and bearing short digitate branches—the whole a compact, intricate apparatus. The basal protions of Figure 1, B and D, represent two such structures of only moderate proportions. The larger examples, which measure more than 200μ in diameter, have a bulk five or ten times as great and are very easily seen with the naked eye. In Figure 1, C and F, are shown still simpler types, while the very simplest, consisting, indeed, of only a single inflated element, is represented in Figure 1, E. These complexes are apparently always borne terminally on a hypha of ordinary diameter, from which, on attaining definitive size,
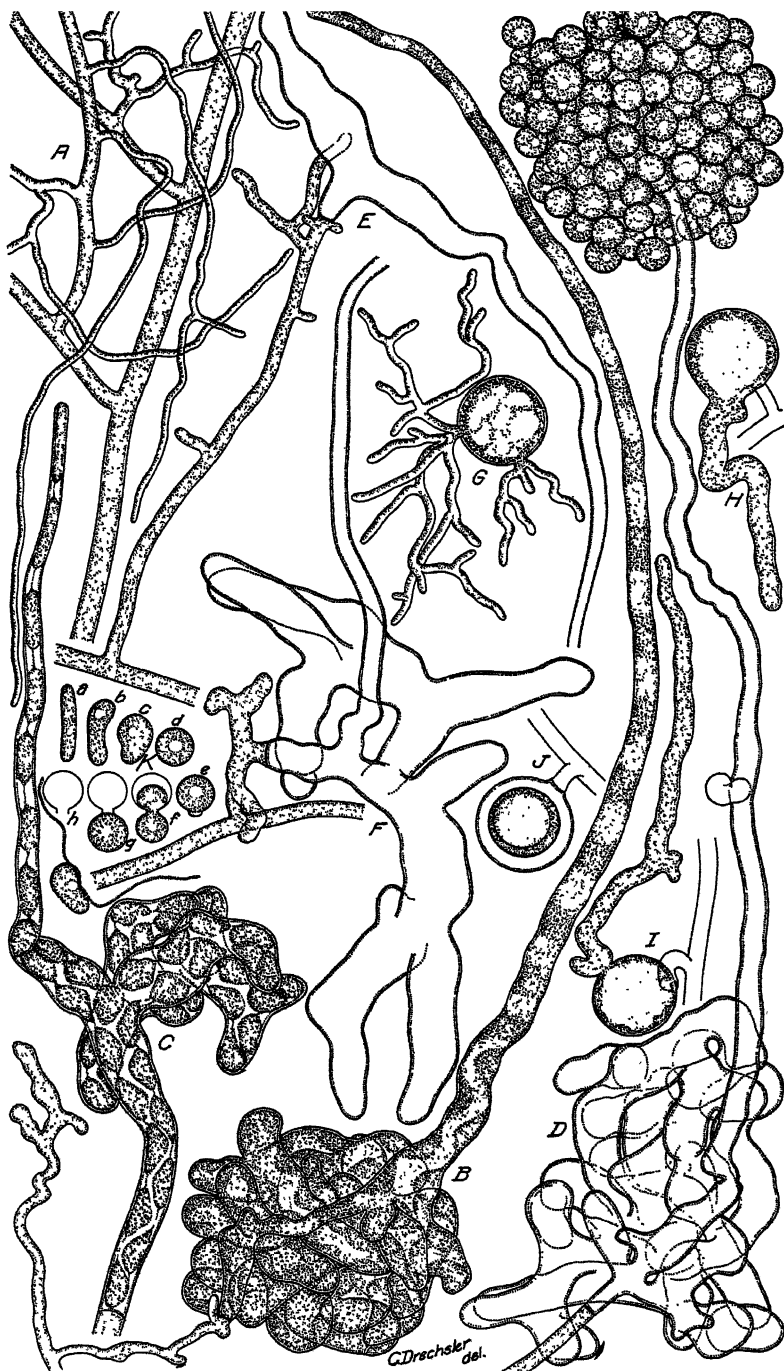
Fig. 1.—Vegetative condition, asexual reproduction, and germination of oospore of *Plectospira myriandra* as occurring in material grown in pure culture on corn-meal agar and transferred to sterile water. All figures drawn with aid of camera lucida, × 500. A, mycelium, the larger hyphae submerged in substratum, the very delicate filaments growing free into surrounding water; B, sporangium showing contents becoming separated into zoospore initials; C, sporangium of small size with zoospores ready for discharge; D, sporangium after evacuation with irregular mass of encysted zoospores at mouth of efferent element; E and F, two small sporangia after discharge; G, oospore in water germinating by delicate hyphae; H and I, oospore embedded in substratum germinating by single stout germ tubes; J, disappearance of oospore wall initiating process of germination; K, a to h, successive stages in rounding up and an encystment of zoospore followed by production of papilla, evacuation of contents, and escape in motile form

they are regularly set off by a fairly thick septum. As their enlargement nears completion, there is produced distally from one of the distended parts—often from one that appears as somewhat of a direct prolongation of the supporting hypha—a filamentous element of diminishing diameter that attains finally a length varying from 100μ to 600μ or 700μ. This filamentous element, which is sometimes marked by abrupt bends at irregular intervals or again may be of regular outline throughout its course, serves in the evacuation of the sporangium as an efferent tube.

Zoospore formation is associated, as in related forms, with protoplasmic changes (fig. 1, B), which in the efferent hypha differs in no particular from that characteristic of the genus Aphanomyces and results here also in a single series of protoplasts connected by delicate strands (fig. 1, C). Within the inflated elements the process results usually in two parallel series of such protoplasts. When the tip of the efferent filament gives way evacuation takes place, often with violent rapidity, at an approximate rate of perhaps 10 zoospores a second, so that scores of these bodies may be observed at the mouth of the tube in their original cylindrical shape, or in scarcely altered early stages of contraction. The total number of zoospores delivered from a single efferent element varies from a minimum of about 20 to 25 to a usual maximum of from 400 to 500, depending on the size of the sporangium involved. The sporangial complexes that exceed at all considerably the proportions of those shown in Figure 1, B and D, usually are provided with plural efferent tubes, each of which functions independently in evacuating portions of the aggregate apparatus, which may thus best be regarded as constituting a compound structure made up of the basal parts of a number of sporangia.

The zoospores remain massed at the mouth of the sporangium in an encysted condition for a period of approximately two hours (fig. 1, D), after which they escape from the cyst wall (fig. 1, K), swim about actively for a time, round up a second time, and under suitable conditions, germinate. The details associated with the discharge of the cyst contents are identical with those described for *Aphanomyces euteiches*, even with respect to the dimensions of the papilla and of the short evacuation tube that persists on the empty cyst wall.

The sexual stage of the fungus develops readily on various media, being produced in quantity, for example, on corn-meal agar. The oogonia appears as subspherical bodies, occasionally intercalary or laterally intercalary in position (fig. 2, D and E) but much more frequently formed terminally on relatively short branches from the stouter hyphae (fig. 2, A–C, F–I). These branches do not generally exceed in length the diameter of the oogonium and frequently measure considerably less. The septum delimiting the oogonium may be inserted nearly tangent to the subspherical part, but more typically is inserted somewhat lower, including, as it were, a portion of the stalk. In the mature parthenogenetic apparatus the oogonial wall often seems lightly sculptured internally, and at times it presents the appearance of numbers of minute, scattered pits. Whether these inconspicuous features are to be homologized with the pronounced pits distinctive of the oogonia of various genera of Saprolegniaceae is not altogether clear, but would seem very doubtful.
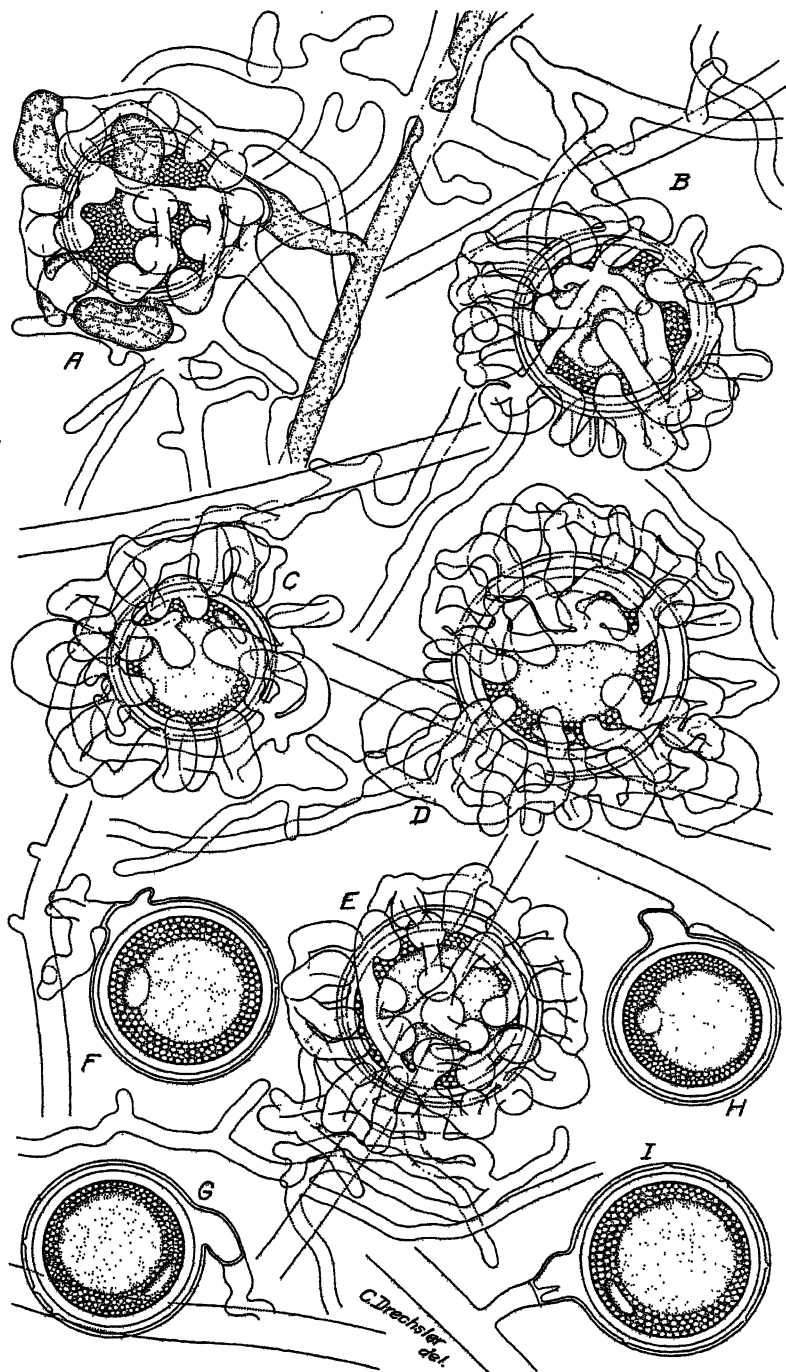
FIG. 2.—Sexual and parthenogenetic apparatus of *Plectospira myriandra* drawn from corn-meal agar cultures with aid of camera lucida, × 1,000. A to E, oogonia invested with numerous antheridial elements, terminal on short branches in A, B, and C; laterally intercalary in D and E (the four antheridia in A with contents shown by stippling are of potentially functional type); F to I, mature parthenogenetic oogonia with oospores

In most cultures from one-half to two-thirds of the eggs are usually produced parthenogenetically.   (Fig. 2, F–I.)   The remainder are formed in the presence of antheridial elements in extraordinary abundance.   (Fig. 2, A–E.)   The elements are uniformly of declinous origin, arising from delicate, sinuous hyphae.   In the proximity of the oogonium one or several of these hyphae put out branches, the resultant filaments enveloping different regions of the female structure and giving off at short intervals, either directly or on short, spurlike branches, elements having the shape and the positional and contact relations usual for antheridia.   Most of these elements never are delimited by septa and soon appear as empty structures in consequence either of the withdrawal or the degeneration of their contents.   In most instances, however, several of the structures, usually terminal in position and often distinguished from the others by greater size, a thicker wall, and more extensive contact with the oogonium, will be found delimited by a septum and filled with dense protoplasmic material.   (Fig. 2, A.)   These structures have all the appearance of true antheridia.   In more advanced material they are, like the rudimentary elements, devoid of contents.

Owing to the difficulty of accurate observation due to the intricacy of the apparatus, clear evidence as to the performance of these organs was not obtained, yet indications that their contents are discharged into the oogonium were not lacking.   In any case, however, the presence of the antheridial elements could scarcely be interpreted as necessary for the development of the egg or as in any way promoting its formation.   Indeed, the egg apparatus provided with them appeared in general to be considerably slower in maturing and slightly more subject to degeneration than the parthenogenetic apparatus, and it was also more likely to be of extreme dimensions. The antheridial elements are remarkable chiefly because they are exceptionally numerous; rarely fewer than 15 and sometimes as many as 35 were visible on the upper side and about the equatorial plane of the oogonium, and perhaps 10 or 20 more may be concealed beneath the oogonium.   The virtual investment of the female organ with such a bristling array of curved structures is one of the striking characteristics of this species.

The oospore is regularly found singly in an oogonium, which it usually occupies almost completely, although there is nearly always a narrow space between the oospore wall and the oogonial wall which occasionally becomes more pronounced in width.   The ripe oospore generally has a wall of moderate thickness, and contains a large spherical homogeneous structure somewhat eccentrically placed in a peripheral matrix.   Within this matrix granulelike bodies of uniform size are arranged geometrically.   They are often 3 or 4 layers deep on one side and 1 or 2 or more rarely 3 layers deep on the other. A strongly refringent homogeneous body oblate ellipsoidal in shape and 5μ or 6μ in diameter by 1.5μ to 2.0μ in thickness, can always be distinguished in the matrix, usually embedded between the outer and inner layer of granular structures, where it appears in profile as an elliptical occlusion.   This body, entirely similar to homologous structures in the oospores of Aphanomyces, Pythium, and related genera, may possibly be the nucleus.

Germination of the oospores readily takes place on transfer to water.   The oospore wall loses its visibility evidently as a result of

some process of gelatinization (fig. 1, J), after which the contents become distributed throughout the interior of the oogonium. A single stout germ tube corresponding in diameter to the larger vegetative hyphae (fig. 1, H and I), or one or several more richly branching systems of hyphae corresponding to the more delicate mycelial elements (fig. 1, G), may be produced, apparently depending largely on whether the oospore is embedded in a matrix (corn-meal agar, in the trials under discussion) or is immediately in contact with water. In a few instances the single germ tube appeared to terminate abruptly in a small lobulate sporangium, making it seem probable that the production of germ sporangia, frequent in *Aphanomyces euteiches*, occurs here occasionally.

<div align="center">TAXONOMY</div>

In the taxonomy of the Saprolegniaceae major importance has been assigned to the type of development exhibited by the zoospores as well as to the morphology of the zoosporangium. With respect to the former feature, the tomato parasite reveals complete similarity to Achlya and Aphanomyces; with respect to the latter, a partial combination of the characteristics of both these genera. In Achlya the zoospores are fashioned within a massive sporangium, usually of tapering-cylindrical shape, regular in outline, such apical modification for evacuation as may be present being generally very slight. Zoospores may be produced also by the gemmae that develop in many species. These bodies frequently occur in irregular forms, as is evidenced, for example, by Coker's figures of *Achlya flagellata* Coker (*2, pl. 37, fig. 7*) and *Achlya megasperma* Humphrey (*2, pl. 44, fig. 9*). Gemmae of this type and other structures, like some of the more irregular "Conidienstände" depicted by Maurizio (*6, Taf. I, figs. 13, 27*), show a certain degree of resemblance in shape to the simpler, more openly disposed sporangia produced by the fungus under consideration. However, even the most copiously branched gemmae of Achlya or, indeed, of any recognized genus of the Saprolegniaceae, fail to exhibit the measure of complexity and involvement found in the better developed sporangia of the fungus discussed in this paper. These latter, moreover, can scarcely be regarded as "Hemmungsbildungen," to which Klebs' (*5*) researches have reduced many of the structures designated as gemmae. Their production is most luxuriant from fresh mycelium that previous to its transfer to water has been well nourished and is not thereafter exposed to serious bacterial contamination—conditions under which development would not ordinarily be arrested. Nor do they require a resting period for zoospore production like the generality of gemmae. They are essentially short-lived bodies that in the absence of conditions suitable for the fashioning and discharging of zoospores soon degenerate, or, at best, proliferate vegetatively after a promiscuous manner.

With respect to the vegetative thallus a pronounced difference between the tomato parasite and Achlya is evident, the mycelium of the former being, of course, decidedly smaller in diameter. On the other hand, the similarity to the thallus of Aphanomyces is striking, not only as to the thickness of the hyphae, but also as to the general appearance under the microscope. This obvious resemblance, together with the uniseriate production of zoospores in the efferent

hyphae, would seem indicative of a close affinity to that genus—an affinity that finds a peculiarly apt parallel in the genus Pythium, assuming for the latter the wider limits more generally adopted. The genus Pythium includes species entirely analogous to Aphanomyces, in which externally undifferentiated filaments serve as sporangia, as well as a series of forms like the widely distributed parasite, *P. aphanidermatum* (Eds.) Fitz., in which sporangia are represented by communicating systems of distended digitate or lobulate elements, often intricately involved—closely similar in composition to those described in this paper. Schröter (*8*) regarded the Pythium types possessing purely filamentous sporangia as sufficiently distinctive to establish a separate genus, Nematosporangium. As has been pointed out, such a disposition might prove profitable if accompanied by the addition of another genus including the forms with lobulate zoosporangia (*3*).

Assignment of the present fungus to Aphanomyces, to which it seems most closely related, could apparently be accomplished only in contravention of the feature most distinctive of that genus. The adoption of a new genus would seem less objectionable, especially as it is not improbable that as more terrestrial types of Saprolegniaceae come to light other forms of similar morphology may require taxonomic treatment. Such a genus is therefore proposed here under a name meaning "plaited coil"; and it is hoped that a further characterization of the fungus may be conveyed in the specific name suggestive of the abundance of antheridial elements.

<center>DIAGNOSIS</center>

Plectospira, n. g.
Mycelium slender, sparingly or moderately branched. Zoosporangia composed of inflated elements, often compacted into an irregular complex, within which zoospores are differentiated in two or more series, together with a prolonged filamentous element within which zoospores are formed in one series and by which the entire organ is evacuated. Zoospores encysting at the mouth of the efferent hypha, later escaping from their cysts and swarming. Oogonia intercalary or terminal. Antheridia absent or present. Oospores single and somewhat eccentric (subcentric) in internal structure.

Plectospira myriandra, n. sp.
Mycelium 1.8μ to 6μ in diameter. Inflated elements of sporangia 6μ to 18μ in diameter; efferent hyphae usually 5μ to 10μ at base, generally tapering more or less to a diameter 3.5μ to 4.5μ at tip. Sporangia sometimes very extensive and compound; then provided with plural efferent hyphae, each delivering up to an approximate maximum of 500 zoospores. Zoospores, after encystment, 6μ to 12μ in diameter, usually 9μ to 10μ, developing a papilla 2.5μ to 3μ in diameter and 1μ long, the cylindrical wall of which after evacuation persists on the empty cyst wall. Oogonium mostly terminal on short branches, more rarely laterally intercalary or intercalary, subspherical, smooth, 15μ to 33μ in diameter, usually 23 to 29μ, provided with a wall generally approximately 0.5μ, more rarely up to 1μ in thickness. Antheridia absent, or frequently 25 to 55 in number, mostly rudimentary, the smallest approximately 3μ in diameter and 5μ in length, often without delimiting septum; the largest, up to 6.5μ in diameter and 25μ in length, delimited by septum and often potentially functional in appearance; mostly straight, distended cylindrical or curved cylindrical; diclinous in origin, borne in close arrangement on a number of branching systems arising from delicate hyphae. Oospore, single, 13μ to 30μ, usually 20μ to 27μ in diameter, provided with a wall 1.1μ to 1.9μ, usually 1.5μ in thickness, slightly eccentric in internal structure. Mildly parasitic, causing discoloration and death of rootlet tips of *Lycopersicum esculentum* Mill. in greenhouse at Arlington Experiment Farm, Rosslyn, Va., in May, 1926.

In the foregoing diagnosis of the genus Plectospira, as generally in the definition of taxonomic groups within the Saprolegniaceae, more stress is laid upon the morphology and development of the structures

concerned in asexual reproduction than upon the morphology of the sexual apparatus. Except for the internal structure of the oospores, the degree of importance to be attributed to the latter will remain problematical until congeneric forms have been discovered. The presence of antheridial elements in such abundance as to envelop the oogonium to a considerable extent, while not common, is far from being unknown among other genera of the Saprolegniaceae, rather extreme conditions of such envelopment being found, according to Von Minden (*7*), in *Achlya prolifera* (Nees) deBary and *Achlya oblongata* deBary. Among the forms with single oospores, some species of Aphanomyces show a certain degree of such envelopment, more, however, because of the relatively large size of the several antheridia present than because of their number.

A condition much more similar to that prevailing in *Plectospira myriandra* was described by de Wildeman (*9*) in an account of a fungus he found parasitic on the oogonia of Characeae and designated as *Achlyopsis entospora*, the type of a new genus. The oogonia were characterized as globose or elliptical, formed terminally on branches, and separated from the thallus by a thick septum often inserted at some distance from the swollen portion. Numerous antheridial filaments derived apparently from several mycelial hyphae sometimes completely invested the oogonium "comme d'un réseau ou d'une couche continue." The abundance of antheridial filaments was held to suggest Achlya, but because of the oospores occurring singly in the oogonia and the development of the latter within the host—two characters which the author regarded as exceptional in the Saprolegniaceae—the fungus was referred to the Peronosporaceae. No sporangial stage was recognized as associated with the parasite. In another portion of the text, however, the author described certain structures also found occurring within the affected oogonia of Characease:

"Comme le montrent nos figures, ces mycéliums internes etaient constitués par un gros filament ramifié, contourné en tous sens et formant une masse pelotonnée plus ou moins volumineuse. De certaines portions de ce thalle coralloïde naissaient des filaments étroits s'enfonçant dans le milieu; d'autres portions naissaient des ramifications, primitivement assez larges, s'amincissant progressivement, traversant la paroi de l'oogone et se prolongeant dans l'eau."

Although de Wildeman recognized these coralloid structures as closely similar to certain mycelia produced by *Plasmopora viticola* (B. and C.) Berl. and De T., a comparison of his figures (9: Pl. XI, figs. 1–4) with Figure 1, B–E, reveals a much more complete similarity. There can, indeed, be little doubt that the "thalles coralloïdes" represent evacuated zoosporangia of the same external morphology as that characteristic of the tomato parasite. Some consideration, therefore, was given to the plausibility of a taxonomic disposition whereby this parasite was to be assigned to de Wildeman's genus appropriately emended by referring the coralloid structures to it and assuming for them the rôle of zoosporangia with Achlya-like development. It appeared best, however, to reject this alternative, as de Wildeman reported among certain other Phycomycetous forms present in the same material as his *Achlyopsis entospora*, all apparently in rather intimate confusion, two new species of Pythium, *P. characearum* and *P. gibbosum*. These species also were based solely

upon their sexual stages. In the absence of information ·to the contrary, the probability is presented of the empty "thalles coralloïdes" representing evacuated lobulate zoosporangia of one or the other of these species of Pythium—a probability quite as strong as that they were associated with *Achlyopsis entospora* and represented zoosporangia that had become evacuated after the manner described in this paper for the tomato parasite. That the species to which the binomial *P. characearum* was applied may be a form with lobulate zoosporangia is made more probable by the description of its mycelium as "très abondant, il forme dans le milieu, des pelotes de filaments très enchevêtrés; il semble porter des grappes d'oosporanges."

The structure of the oospore of *Achlyopsis entospora*, with its very thick wall, and its more or less homogeneous contents containing 1 to 4 refringent globules, suggesting in these details little similarity to the structure of the oospore of the fungus affecting tomato rootlets, provides an additional reason for not referring the latter to de Wildeman's genus. As, however, the figures given by that author indicate that his material may have suffered considerable degeneration, it would be inadvisable to attribute too much significance to a comparison of such features.

SUMMARY

Two water molds have been found causing discoloration and· death of occasional rootlet tips of tomato seedlings in greenhouses. One was identified as *Aphanomyces euteiches*, known previously as a cause of root rot of peas. The other is described as the type of a new genus resembling Aphanomyces in general habit and in zoospore development but differing from it in having a differentiated zoosporangium composed typically of an involved complex of inflated elements, and a filamentous efferent hypha.

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THE VALUE OF BEEF PROTEIN AS A SUPPLEMENT TO THE PROTEINS IN CERTAIN VEGETABLE PRODUCTS[1]

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INTRODUCTION

The nutritive value of a protein when fed as the single source of nitrogen in the diet is not necessarily the same as when it is fed in combination with other proteins. If a protein supplies adequate proportions of all the amino acids required by the body, the nutritive value of such a protein will not be enhanced by combining it with other proteins; but, on the other hand, if a protein is deficient in one or more of the essential amino acids, its nutritive value will be greatly increased by feeding it in combination with other proteins which furnish an excess of these acids. To combine proteins in the dietary in such a way as to supply a mixture of high biological value is a matter of very practical importance.

Most meat proteins have a high biological value, but the proteins in the vegetable products which constitute a large part of our dietary are more or less deficient in the essential amino acids. The purpose of the investigation herein reported was to determine the extent to which the protein in beef supplements that in wheat, bolted wheat flour, corn meal, oatmeal, rice, navy beans, and potatoes.

PREVIOUS INVESTIGATIONS

Hoagland and Snider (*1, 2*)[2] studied the nutritive value of the protein in the lean meat and in most of the important edible organs of cattle, calves, sheep, and hogs by means of quantitative feeding experiments with young albino rats. They found that, with the exception of a few products of minor importance, the meats and meat products contained protein of high biological value. Previous investigations concerning the nutritive value of meat proteins are discussed in those papers.

The nutritive value of the protein in wheat and in bolted wheat flour has been determined by Osborne and Mendel (*8, p. 600–601*). They state:

The quantity of protein furnished by the *entire* wheat kernel which is necessary for continued maintenance of adult rats is greater than that required when proteins of milk or of various other food products are fed. For the normal growth of the young this difference is much more pronounced.

For maintaining adults the proteins of the endosperm are adequate; they are inadequate for growth. Additions of meat, milk, or eggs to wheat flour so greatly

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Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 4
Feb. 15, 1927
Key No. A-115

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enhance the value of the protein for growth that a great economy in consumption of protein results. Under the ordinary human dietary conditions the proteins of wheat are advantageously employed.

In another paper the same authors (*9*, *p. 293*) report the results of a study of the nutritive value of the proteins of the barley, oat, rye, and wheat kernels. They conclude that "until a far greater refinement of the method is secured it would appear, on the whole, that the proteins of the four cereals studied are not widely different in their efficiency in promoting growth."

McCollum, Simmonds, and Parsons (*4*) report that the proteins of kidney, liver, and muscle are remarkably valuable for supplementing the proteins of some of the cereals, but that they are less satisfactory as supplements of the legume proteins. They state also that the proteins of kidney, liver, or muscle are more valuable for transformation into body tissue when combined with cereal proteins than when each is fed as the sole source of amino acids in the diet.

McCollum and Simmonds (*3*, *p. 129, 131, 133, 135*) place a high value on the proteins of the entire wheat kernel. They state that wheat protein is slightly more effective in promoting growth than the same amount of protein from milk or eggs. However, they also state that feeding tests in which wheat was the sole source of protein in the diet were not uniformly successful and that apparently some wheats are superior to others. Bolted wheat flour is reported to contain protein of poor quality. Maize, when fed as the sole source of protein in the diet, is considered to have a somewhat lower nutritive value than wheat, and rice is reported to contain proteins of low nutritive value.

According to Sherman (*11*, *p. 247, 231*) the proteins of meat adequately supplement those of grains in nutrition. Wheat, maize, and oat proteins were found to have practically the same value in human nutrition.

Mitchell (*5*) determined the biological value of the protein in a number of food products by means of feeding tests with rats. When the rats were fed at the 5 per cent level the following biological values were obtained: 93.4 for milk, 72 for corn, 78.6 for oats, 86.1 for rice, and 68.5 for potatoes. When the rats were fed at the 10 per cent level, the values were 59.6 for corn, 64.9 for oats, 66.7 for potatoes, and 38.4 for navy beans. These figures refer to digestible protein.

Mitchell and Carman (*6*) report the biological values obtained for the protein in certain food products when fed at an 8 per cent level of intake. Whole wheat had a value of 67, pork 74, and eggs 93.

In a recent publication Mitchell and Carman (*7*) report the biological values obtained for the nitrogen in mixtures of patent white flour and certain animal products. When two parts of white-flour protein were fed in combination with one part of protein from beef, veal, eggs, or milk, the biological value of the protein in the white flour was very materially enhanced. The protein in the mixture of beef and white flour or veal and white flour had as high a biological value as did either beef or veal alone, whereas white flour alone had a much lower value. The supplemental relationship between the proteins in the products tested was found to be greatest between white flour and beef and least between white flour and egg albumin. No distinct difference between the other animal products appeared to exist.

EXPERIMENTAL WORK

PROCEDURE

The methods employed in this investigation are essentially the same as those followed by the writers in previous studies of the nutritive value of the protein in various animal tissues (*1, 2*). Each ration was fed to six young male albino rats for a period of 60 days. The rats were housed in individual cages and an accurate record was kept of the feed consumed. The rats weighed approximately 40 grams each at the beginning of the experiment and they were weighed twice weekly during the tests.

Each ration was made up to contain 10 per cent protein [3] (Nx6.25), exclusive of that in the vitamin B; 10 per cent fat, of which 2 per cent was cod-liver oil as a source of vitamins A and D; 4 per cent ash mixture; 2 per cent vitamin B from yeast; and the rest cassava starch, which has been found to be practically free from nitrogen. The vitamin B product contained approximately 50 per cent protein so that about 1 per cent of crude protein from this source was added to the ration.

DESCRIPTION OF PRODUCTS TESTED

The beef consisted of round steak from high-grade beef. The muscle was trimmed as free from fatty and connective tissues as practicable, ground, mixed with water and toluol, and dried in a current of air at 60° C. Several lots of beef were used in the experiments.

Three lots of yellow corn meal, two lots each of hard spring wheat, northwestern fancy patent flour, oatmeal, and potatoes, and one lot each of ordinary commercial rice and navy beans were used. The navy beans were soaked overnight, boiled until soft, ground, and dried in a current of air. The potatoes were peeled, boiled until thoroughly cooked, ground, and dried in a current of air. All the products named above, except the flour and corn meal, were ground to fine condition before they were mixed in the rations.

RESULTS OF EXPERIMENTS WITH RATIONS CONTAINING 10 PER CENT OF ANIMAL OR VEGETABLE PROTEIN

In Table 1 are reported the results of the 30-day experiments with rations containing 10 per cent each of protein from beef, pork, lamb, wheat, bolted wheat flour, oatmeal, and navy beans, respectively. Since there are greater or lesser variations in the results obtained from the individual rats fed a single ration, judgment of the apparent relative nutritive value of the protein in the different products should be based upon the average data for each group of rats.

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[3] The writers have found that 10 per cent of beef protein in a ration containing 10 per cent of fat is somewhat less protein than is required for optimal growth in young rats during a period of 60 days. Five per cent of beef protein is adequate only for maintenance.

TABLE 1.—*Comparative nutritive values of animal and vegetable proteins when fed at the 10 per cent level for 30 days to young male albino rats*

Source of protein	Rat No.	Age at begin- ning of test	Initial weight	Gain in weight in 30 days	Total intake Feed	Total intake Protein	Intake per gram gain in weight Feed	Intake per gram gain in weight Protein	Gain in weight per gram Feed	Gain in weight per gram Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Ox muscle (beef)	422 A	32	48	77	279	27.9	3.6	0.36	0.28	2.76
Do	422 B	32	51	113	359	35.9	3.2	.32	.31	3 15
Do	422 D	32	49	98	302	30.2	3.1	.31	.32	3.24
Do	588 D	26	38	92	268	26.8	2.9	.29	.34	3 43
Average		31	47	95	302	30.2	3.2	.32	.31	3 15
Hog muscle (pork)	384 C	24	48	84	281	28.1	3.3	.33	.30	2.99
Do	384 D	24	46	86	288	28.8	3.3	.33	.30	2.99
Do	570 A	24	40	66	250	25.0	3.8	.38	.26	2 64
Do	570 B	24	40	98	305	30.5	3.1	.31	.32	3.21
Average		24	44	84	281	28.1	3.4	.34	.30	2.96
Sheep muscle (lamb)	444 B	25	39	87	289	28.9	3.3	.33	.30	3.01
Do	571 A	24	40	84	266	26.6	3.2	.32	.32	3.16
Do	571 B	24	42	93	274	27.4	2.9	.29	.34	3.39
Do	571 C	24	41	77	265	26.5	3.4	.34	.29	2.91
Average		24	41	85	274	27.4	3.2	.32	.31	3.12
Wheat	720 A	26	45	39	180	18.0	4.6	.46	.22	2.17
Do	720 B	26	44	39	171	17.1	4.4	.44	.23	2.28
Do	720 C	26	45	33	171	17.1	5 2	.52	.19	1.93
Do	720 D	22	43	37	200	20.0	5 4	.54	.19	1.85
Do	720 E	22	41	41	186	18.6	4.5	.45	.22	2 20
Do	720 F	23	41	37	182	18.2	4.9	.49	.20	2.03
Average		24	43	38	182	18.2	4.8	.48	.21	2.08
Wheat flour (patent)	722 A	30	40	17	154	15.4	9.0	.90	.11	1.10
Do	722 B	26	41	27	178	17.8	6.6	.66	.15	1.52
Do	722 C	24	38	18	139	13.9	7 7	.77	.13	1.30
Do	722 D	24	40	21	180	18.0	8.6	.86	.12	1.17
Do	722 E	23	39	15	137	13.7	9.1	.91	.11	1.09
Do	722 F	23	40	19	139	16.3	7.3	.73	.14	1.37
Average		25	40	20	155	15.5	8.1	.81	.13	1.27
Oatmeal	710 A	28	44	46	200	20.0	4.3	.43	.23	2.30
Do	710 B	28	41	45	171	17.1	3.8	.38	.26	2.63
Do	710 C	25	43	55	230	23.0	4.2	.42	.24	2.39
Do	710 D	25	38	29	183	18.3	6.3	.63	.16	1.58
Do	710 E	28	48	31	179	17.9	5.8	.58	.17	1.73
Do	710 F	27	40	43	170	17.0	4.0	.40	.25	2 53
Average		27	42	42	189	18.9	4.7	.47	.22	2.19
Navy beans	752 A	23	38	32	191	19.1	6.0	.60	.17	1.67
Do	752 B	23	39	35	211	21.1	6.0	.60	.17	1.66
Do	752 C	23	38	10	135	13.5	13.5	1.35	.07	.74
Do	752 D	23	40	24	181	18.1	7.5	.75	.13	1.33
Do	752 E	23	40	39	211	21.1	5.4	.54	.18	1.85
Do	752 F	28	44	31	188	18.8	6.1	.61	16	1.65
Average		24	40	29	186	18.6	7.4	.74	.15	1.48

The data in Table 1 show very clearly that the protein in beef, pork, and lamb was very much more efficient for maintenance and growth of albino rats when fed at the 10 per cent level for 30 days than was the protein in wheat, bolted wheat flour, oatmeal, or navy beans. The protein in wheat and in oatmeal had approximately the same nutritive value, followed in turn by that in navy beans and bolted wheat flour. The gain in weight per gram of protein consumed ranged from 2.96 to 3.15 grams for the rats fed the meat proteins as compared with 1.23 to 2.19 grams for those fed the vegetable proteins. The rats fed meat proteins made an average gain in weight of 88 grams during the 30-day test, whereas the best average gain made by those fed any one of the vegetable proteins was only 42 grams.

In Table 2 are reported the results of the 60-day feeding tests with rations containing 10 per cent each of meat or vegetable protein. Again the meat proteins were very much more efficient in promoting growth than the vegetable proteins. Among the vegetable products oatmeal seemed to be a slightly better source of protein than wheat, while bolted wheat flour and navy beans were considerably less satisfactory. The rats getting meat protein made an average gain in weight of 153 grams during the 60 days of the experiment, whereas those fed vegetable proteins made gains ranging from 41 grams for bolted wheat flour to 82 grams for oatmeal. The rats fed meat protein gained, on an average, 2.5 grams in weight for each gram of protein consumed, but the rats fed vegetable proteins made gains ranging only from 1.16 grams for bolted wheat flour to 1.89 grams for oatmeal.

TABLE 2.—*Comparative nutritive values of animal and vegetable proteins when fed at the 10 per cent level for 60 days to young male albino rats*

Source of protein	Rat No.	Age at begin-ning of test	Initial weight	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Ox muscle (beef)___	422 A	32	48	146	603	60.3	4.1	0.41	0.24	2.42
Do_____	422 B	32	51	189	776	77.6	4.1	.41	.24	2.44
Do_____	422 D	32	49	172	687	68.7	4.0	.40	.25	2.50
Do_____	588 D	26	38	171	601	60.1	3.5	.35	.28	2.85
Average_____	--------	31	47	170	667	66.7	3.9	.39	.25	2.55
Hog muscle (pork)__	384 C	24	48	127	582	58.2	4.6	.46	.22	2.18
Do_____	384 D	24	46	147	550	55.0	3.7	.37	.27	2.67
Do_____	570 A	24	40	111	485	48.5	4.4	.44	.23	2.29
Do_____	570 B	24	40	163	600	60.0	3.7	.37	.27	2.72
Average_____	--------	24	44	137	554	55.4	4.1	.41	.25	2.46
Sheep muscle (lamb)	444 B	25	39	152	656	65.6	4.3	.43	.23	2.32
Do_____	571 A	24	40	157	596	59.6	3.8	.38	.26	2.63
Do_____	571 B	24	42	162	616	61.6	3.8	.38	.26	2.63
Do_____	571 C	24	41	135	575	57.5	4.3	.43	.23	2.35
Average_____	--------	24	41	152	611	61.1	4.1	.41	.25	2.48
Wheat_____	720 A	26	45	81	462	46.2	5.7	.57	.18	1.75
Do_____	720 B	26	44	73	418	41.8	5.7	.57	.17	1.75
Do_____	720 C	26	45	67	418	41.8	6.2	.62	.16	1.60
Do_____	720 D	22	43	74	479	47.9	6.5	.65	.15	1.54
Do_____	720 E	22	41	69	431	43.1	6.2	.62	.16	1.60
Do_____	720 F	23	41	60	476	47.6	7.9	.79	.13	1.26
Average_____	--------	24	43	71	447	44.7	6.4	.64	.16	1.58
Wheat flour (patent)	722 A	30	40	39	356	35.6	9.1	.91	.11	1.10
Do_____	722 B	26	41	51	398	39.8	7.8	.78	.13	1.28
Do_____	722 C	24	38	43	328	32.8	7.6	.76	.13	1.31
Do_____	722 D	24	40	46	394	39.4	8.6	.86	.12	1.17
Do_____	722 E	23	39	28	285	28.5	10.2	1.02	.10	.98
Do_____	722 F	23	40	39	351	35.1	9.0	.90	.11	1.11
Average_____	--------	25	40	41	352	35.2	8.7	.87	.12	1.16
Oatmeal_____	710 A	28	44	78	442	44.2	5.7	.57	.18	1.76
Do_____	710 B	28	41	91	412	41.2	4.5	.45	.22	2.21
Do_____	710 C	25	43	119	559	55.9	4.7	.47	.21	2.13
Do_____	710 D	25	38	34	315	31.5	9.3	.93	.11	1.08
Do_____	710 E	28	48	81	423	42.3	5.2	.52	.19	1.91
Do_____	710 F	27	40	88	395	39.5	4.5	.45	.22	2.23
Average_____	--------	27	42	82	424	42.4	5.7	.57	.19	1.89
Navy beans_____	752 A	23	38	45	430	43.0	9.6	.96	.10	1.05
Do_____	752 B	23	39	67	462	46.2	6.9	.69	.15	1.45
Do_____	752 C	23	38	35	293	29.3	8.4	.84	.12	1.19
Do_____	752 D	23	40	57	399	39.9	7.0	.70	.14	1.43
Do_____	752 E	23	40	62	420	42.0	6.8	.68	.15	1.48
Do_____	752 F	28	44	50	376	37.6	7.5	.75	.13	1.33
Average_____	--------	24	40	53	397	39.7	7.7	.77	.13	1.32

RESULTS OF EXPERIMENTS WITH RATIONS CONTAINING 5 PER CENT EACH OF ANIMAL AND OF VEGETABLE PROTEIN

In Table 3 are reported the results of the 30-day experiments with rations containing each 5 per cent of beef protein and 5 per cent of protein from wheat, bolted wheat flour, oatmeal, corn meal, rice, navy beans, and potatoes, respectively.

TABLE 3.—*Comparative nutritive values of mixtures of equal parts of beef and vegetable proteins when fed at the 10 per cent level for 30 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef and wheat	721 A	24	44	108	305	30.5	2 8	0.28	0.35	3.54
Do	721 B	28	45	97	289	28 9	3.0	.30	.34	3.36
Do	721 C	27	39	94	285	28.5	3.0	30	.33	3.30
Do	721 D	28	39	80	256	25.6	3.2	.32	.31	3.12
Do	721 E	28	41	68	236	23.6	3.5	.35	.29	2.88
Do	721 F	28	43	92	294	29.4	3.2	.32	.31	3.13
Average		27	42	90	278	27 8	3 1	.31	.32	3.22
Beef and wheat flour (patent)	723 A	23	39	69	229	22 9	3.3	.33	.30	3.01
Do	723 B	23	39	67	239	23.9	3.6	.36	.28	2.80
Do	723 C	24	39	78	247	24.7	3.2	.32	.32	3 16
Do	723 D	28	41	76	259	25 9	3.4	.34	.29	2.93
Do	723 E	24	40	64	234	23.4	3.7	.37	.27	2.74
Do	723 F	24	37	79	259	25.9	3 3	.33	.31	3.05
Average		24	39	72	245	24.5	3.4	.34	.30	2.95
Beef and corn meal	716 A	26	38	96	266	26.6	2.8	.28	.36	3.61
Do	716 B	26	39	66	231	23.1	3.5	.35	.29	2.86
Do	716 C	30	39	75	246	24 6	3.3	.33	.30	3.04
Do	716 D	30	44	71	254	25.4	3.6	.36	.28	2.80
Do	716 E	24	44	123	340	34.0	2.8	.28	.36	3 62
Do	716 F	24	44	96	316	31.6	3.3	.33	.30	3.04
Average		27	41	88	276	27 6	3.2	.32	.32	3.16
Beef and oatmeal	711 A	26	43	65	214	21 4	3.3	.33	.30	3 04
Do	711 B	26	41	84	247	24.7	2.9	.29	.34	3.40
Do	711 C	26	42	92	325	32.5	3.5	.35	.28	2 83
Do	711 D	26	38	74	245	24 5	3.3	.33	.30	3.02
Do	711 E	26	44	94	297	29.7	3.2	.32	.32	3 16
Do	711 F	27	41	95	286	28.6	3.0	.30	.33	3.32
Average		26	42	84	269	26.9	3.2	.32	.31	3 13
Beef and rice	749 A	27	39	110	310	31.0	2.8	.28	.35	3.55
Do	749 B	25	44	120	362	36.2	3.0	.30	.33	3.31
Do	749 C	25	46	110	343	34.3	3.1	.31	.32	3.21
Do	749 D	25	44	120	352	35.2	2.9	.29	.34	3.41
Do	749 E	25	41	104	330	33.0	3 2	.32	.32	3.15
Do	749 F	28	39	115	338	33.8	2.9	.29	.34	3.40
Average		26	42	113	339	33.9	3.0	.30	.33	3.34
Beef and navy beans	753 A	25	39	44	196	19.6	4.5	.45	.22	2.24
Do	753 B	26	39	49	209	20.9	4.3	.43	.23	2.34
Do	753 C	26	40	59	253	25.3	4.3	.43	.23	2.33
Do	753 D	27	40	49	223	22.3	4.6	.46	.22	2.19
Do	753 E	27	39	59	230	23.0	3.9	.39	.26	2.57
Do	753 F	27	39	43	192	19.2	4.5	.45	.22	2.24
Average		26	39	51	217	21.7	4.4	.44	.23	2.32
Beef and potatoes	766 A	27	45	23	150	15.0	6.5	.65	.15	1.53
Do	766 B	27	45	26	149	14.9	5.7	.57	.17	1 74
Do	766 C	27	43	21	141	14.1	6.7	.67	.15	1.49
Do	766 D	24	42	34	163	16.3	4.8	.48	.21	2.09
Do	766 E	24	43	29	153	15.3	5.3	.53	.19	1.89
Do	766 F	24	41	31	152	15.2	4 9	.49	.20	2.04
Average		26	43	27	151	15.1	5.7	.57	.18	1.80

The results of these experiments indicate that beef protein very greatly enhances the nutritive value of the protein in our four most important food cereals when the two kinds of protein are mixed in equal proportions in the diet. The rations containing wheat, bolted wheat flour, corn meal, oatmeal, and rice, respectively, were approximately of equal value in promoting growth, and this value was about the same as that previously obtained for rations containing the same percentage of meat protein alone. (Table 1.) On the other hand, the rations containing beef and navy beans or potatoes had much lower values.

Among the rats fed beef and cereal rations, the gain in weight per gram of protein consumed during the 30-day test ranged from 2.95 grams for those fed the beef-wheat flour ration to 3.34 grams for those fed the beef-rice ration, as compared with an average gain of 3.08 grams for those fed the rations containing meat protein alone.

· In Table 4 are reported the results of the 60-day tests with rations containing equal parts of beef and vegetable proteins. As in the 30-day feeding experiments with the same rations, these tests show that the rations containing equal parts of beef and cereal proteins were practically of the same value in promoting growth in rats as rations containing only meat protein. The rations containing equal parts of beef and bean or potato protein, however, were of much less value, as in the 30-day experiment.

TABLE 4.—*Comparative nutritive values of mixtures of equal parts of beef and vegetable proteins when fed at the 10 per cent level for 60 days to young male albino rats*

Source of protein	Rat No.	Age at begin-ning of test	Initial weight	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Pro-tein	Feed	Pro-tein	Feed	Pro-tein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef and wheat	721 A	24	44	173	695	69.5	4.0	0.40	0.25	2.49
Do	721 B	28	45	125	601	60.1	4.8	.48	.21	2.08
Do	721 C	27	39	154	662	66.2	4.3	.43	.23	2.33
Do	721 D	28	39	139	617	61.7	4.4	.44	.23	2.25
Do	721 E	28	41	118	537	53.7	4.6	.46	.22	2.20
Do	721 F	28	43	140	624	62.4	4.5	.45	.22	2.24
Average		27	42	142	623	62.3	4.4	.44	.23	2.27
Beef and wheat flour (patent)	723 A	23	39	162	590	59.0	3.6	.36	.27	2.75
Do	723 B	23	39	154	594	59.4	3.8	.38	.26	2.59
Do	723 C	24	39	155	588	58.8	3.8	.38	.26	2.64
Do	723 D	28	41	119	538	53.8	4.5	.45	.22	2.21
Do	723 E	24	40	143	603	60.3	4.2	.42	.24	2.37
Do	723 F	24	37	158	613	61.3	3.9	.39	.26	2.58
Average		24	39	149	588	58.8	3.9	.38	.25	2.53
Beef and corn meal	716 A	26	38	165	624	62.4	3.8	.38	.26	2.64
Do	716 B	26	39	123	539	53.9	4.4	.44	.23	2.28
Do	716 C	30	39	147	599	59.9	4.1	.41	.25	2.45
Do	716 D	30	44	140	616	61.6	4.4	.44	.23	2.26
Do	716 E	24	44	195	756	75.6	3.9	.39	.26	2.58
Do	716 F	24	44	182	760	76.0	4.2	.42	.24	2.40
Average		27	41	159	649	64.9	4.1	.41	.25	2.44
Beef and oatmeal	711 A	26	43	161	577	57.7	3.6	.36	.28	2.79
Do	711 B	26	41	159	610	61.0	3.8	.38	.26	2.61
Do	711 C	26	42	143	678	67.8	4.7	.47	.21	2.11
Do	711 D	26	38	158	637	63.7	4.0	.40	.25	2.48
Do	711 E	26	44	185	715	71.5	3.9	.39	.26	2.59
Do	711 F	27	41	175	697	69.7	4.0	.40	.25	2.51
Average		26	42	164	652	65.2	4.0	.40	.25	2.52
Beef and rice	749 A	27	39	189	707	70.7	3.7	.37	.27	2.67
Do	749 B	25	44	195	773	77.3	4.0	.40	.25	2.52
Do	749 C	25	46	173	729	72.9	4.2	.42	.24	2.37
Do	749 D	25	44	201	749	74.9	3.7	.37	.27	2.68
Do	749 E	25	41	174	719	71.9	4.1	.41	.24	2.42
Do	749 F	28	39	167	704	70.4	4.2	.42	.24	2.37
Average		26	42	183	730	73.0	4.0	.40	.25	2.50
Beef and navy beans	753 A	25	39	102	501	50.1	4.9	.49	.20	2.04
Do	753 B	26	39	87	447	44.7	5.1	.51	.19	1.95
Do	753 C	26	40	111	563	56.3	5.1	.51	.20	1.97
Do	753 D	27	40	103	497	49.7	4.8	.48	.21	2.07
Do	753 E	27	39	120	563	56.3	4.7	.47	.21	2.13
Do	753 F	27	39	88	441	44.1	5.0	.50	.20	2.00
Average		26	39	102	502	50.2	4.9	.49	.20	2.03
Beef and potatoes	766 A	27	45	57	337	33.7	5.9	.59	.17	1.69
Do	766 B	27	45	65	356	35.6	5.5	.55	.18	1.83
Do	766 C	27	43	56	335	33.5	6.0	.60	.17	1.67
Do	766 D	24	42	88	430	43.0	4.9	.49	.20	2.05
Do	766 E	24	43	72	364	36.4	5.1	.51	.20	1.98
Do	766 F	24	41	63	346	34.6	5 5	.55	.18	1.82
Average		26	43	67	361	36.1	5.5	.55	.18	1.84

RESULTS OF EXPERIMENTS WITH RATIONS CONTAINING 6.67 PER CENT OF VEGETABLE PROTEIN AND 3.33 PER CENT OF ANIMAL PROTEIN

In Table 5 are reported the results of the 30-day experiments with rations containing two parts of vegetable protein and one part of animal protein. In these experiments the rations containing beef and cornmeal or oatmeal induced appreciably greater and more economical growth then the rations containing beef and wheat or wheat flour. The rations containing beef and cornmeal or oatmeal were fully as efficient in promoting growth during a 30-day test as were rations containing a like percentage of beef protein alone. (Table 1.) Rice, on account of its low protein content, could not be used in a ration in sufficient quantity to furnish two parts of protein to one part of beef protein.

TABLE 5.—*Comparative nutritive values of mixtures of one-third beef protein and two-thirds vegetable protein when fed at the 10 per cent level for 30 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef and wheat	756 A	25	41	83	260	26.0	3.1	0.31	0.32	3.19
Do	756 B	25	42	75	269	26.9	3.6	.36	.28	2.79
Do	756 C	25	40	87	299	29.9	3.4	.34	.29	2.91
Do	756 D	25	45	69	264	26.4	3.8	.38	.26	2.61
Do	756 E	25	45	60	253	25.3	4.2	.42	.24	2.37
Do	756 F	25	43	48	217	21.7	4.5	.45	.22	2.21
Average		25	43	70	260	26.0	3.8	.38	.27	2.68
Beef and wheat flour (patent)	765 A	24	40	55	216	21.6	3.9	.39	.25	2 55
Do	765 B	24	39	65	243	24.3	3.7	.37	.27	2.67
Do	765 C	24	44	72	253	25.3	3.5	.35	.28	2.84
Do	765 D	24	39	56	227	22.7	4.1	.41	.25	2.47
Do	765 E	23	39	78	285	28.5	3.7	.37	.27	2.74
Do	765 F	23	42	77	284	28.4	3.7	.37	.27	2.71
Average		24	41	67	251	25.1	3.8	.38	.27	2.67
Beef protein 4 per cent and cornmeal protein 6 per cent	770 A	28	38	100	338	33.8	3.4	.34	.29	3.96
Do	770 B	28	43	153	452	45.2	3.0	.30	.34	3.38
Do	770 C	25	41	107	364	36 4	3.4	.34	.29	2.94
Do	770 D	25	38	66	269	26.9	4.1	.41	.25	2.45
Do	770 E	25	39	87	311	31.1	3.6	.36	.28	2.80
Do	770 F	25	40	105	339	33.9	3.2	.32	.31	3.10
Average		26	40	103	346	34.6	3.5	.35	.29	2.94
Beef and oatmeal	771 A	24	40	114	321	32.1	2.8	.28	.36	3.55
Do	771 B	24	42	92	298	29.8	· 3.2	.32	.31	3.09
Do	771 C	27	46	95	304	30.4	3.2	.32	.31	3.12
Do	771 D	28	40	101	275	27.5	2.7	.27	.37	3.67
Do	771 E	28	42	68	233	23.3	3.4	.34	.29	2.92
Do	771 F	25	41	79	252	25.2	3.2	.32	.31	3.13
Average		26	42	92	281	28.1	3.1	.31	.33	3.25

In Table 6 are given the results of the 60-day experiments with the rations used in the preceding test. The rations containing beef and wheat flour, corn meal, or oatmeal induced approximately the same growth as rations containing the same percentage of meat protein alone. (Table 2.) The beef and wheat ration, however, was of slightly lower value.

TABLE 6.—*Comparative nutritive values of mixtures of one-third beef protein and two-thirds vegetable protein when fed at the 10 per cent level for 60 days to young male albino rats*

Source of protein	Rat No.	Age at beginning of test	Initial weight	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight per gram	
					Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef and wheat	756 A	25	41	154	622	62.2	4.0	0.40	0.25	2 48
Do	756 B	25	42	154	641	64.1	4.2	.42	.24	2.40
Do	756 C	25	40	161	688	68.8	4.3	.43	.23	2.34
Do	756 D	25	45	149	632	63 2	4.2	.42	.24	2.36
Do	756 E	25	45	119	575	57.5	4.8	.48	.21	2.07
Do	756 F	25	43	89	480	48 0	5.4	.54	.19	1.85
Average		25	43	138	606	60.6	4.5	.45	.23	2 25
Beef and wheat flour (patent)	765 A	24	40	131	516	51.6	3.9	.39	.25	2.54
Do	765 B	24	39	147	597	59.7	4.1	.41	.25	2.46
Do	765 C	24	44	155	604	60.4	3.9	.39	.26	2.57
Do	765 D	24	39	120	503	50.3	4.2	.42	.24	2.39
Do	765 E	23	39	166	688	68 8	4.1	.41	.24	2.41
Do	765 F	23	42	177	684	68.4	3.9	.39	.26	2.59
Average		24	41	149	599	59.9	4.0	40	.25	2.49
Beef protein 4 per cent and cornmeal protein 6 per cent	770 A	28	38	189	762	76.2	4 0	.40	.25	2.48
Do	770 B	28	43	273	952	95.2	3.5	.35	.29	2 87
Do	770 C	25	41	182	721	72.1	3.9	.39	.25	2.52
Do	770 D	25	38	146	565	56.5	3 9	.39	.26	2.58
Do	770 E	25	39	168	726	72.6	4.3	.43	.23	2.31
Do	770 F	25	40	193	742	74.2	3 8	.38	.26	2 60
Average		26	40	192	745	74.5	3.9	39	.26	2.56
Beef and oatmeal	771 A	24	40	201	710	71.0	3 5	.35	.28	2.83
Do	771 B	24	42	166	634	63.4	3.8	.38	.26	2.62
Do	771 C	27	46	188	692	69.2	3.7	.37	.27	2.72
Do	771 D	28	40	194	648	64 8	3.3	33	30	2.99
Do	771 E	28	42	130	555	55.5	4.3	43	.23	2.34
Do	771 F	25	41	126	558	55.8	4.4	.44	.23	2.26
Average		26	42	168	633	63.3	3.8	.38	.26	2 63

DISCUSSION AND SUMMARY

In this paper are reported the results of a series of quantitative feeding experiments with young male albino rats to determine the value of the protein in beef as a supplement to that in wheat, bolted wheat flour, corn meal, oatmeal, rice, navy beans, and potatoes. Each ration contained 10 per cent of protein exclusive of approximately 1 per cent in vitamin B from yeast.

When each product was the only source of protein in the diet, beef had a much higher value for stimulating growth than any of the vegetable products; wheat and oatmeal had approximately the same value; navy beans and bolted wheat flour had considerably lower values.

When beef protein was mixed in equal proportions with vegetable protein, the rations containing the following vegetable products had approximately the same efficiency in promoting growth, viz, wheat, bolted wheat flour, corn meal, oatmeal, and rice. Each of these mixtures also had approximately the same value for promoting growth as a ration containing the same percentage of beef protein

alone. The rations containing equal parts of beef and navy-bean or potato protein had much lower values.

When one part of beef protein was mixed with two parts of vegetable protein, the rations containing the following vegetable products had approximately the same value for inducing growth, viz, wheat, bolted wheat flour, corn meal, and oatmeal. Each of these mixtures also had approximately the same value for inducing growth as a ration containing the same percentage of beef protein alone.

It is interesting to note that, although the entire wheat kernel furnished protein considerably more efficient in promoting growth than did bolted wheat flour, when each product was the only source of protein in the diet, this difference was no longer apparent if either wheat or bolted wheat flour was fed in combination with beef protein. These facts indicate that bolted wheat flour is probably as good a source of protein in the human dietary as flour made from the entire wheat kernel, provided a reasonable amount of beef or other animal protein of high biological value is included in the dietary.

The high supplemental value which the protein in beef, as well as in many other animal products, has for the protein in the cereals is of great practical importance in human nutrition. According to Pearl (*10, p. 226*), the average percentage amounts of protein consumed in this country during the period 1911–12 to 1916–17 were obtained from the following sources: Grains, 36; meats, 26; dairy products, 20; poultry and eggs, 7; vegetables, 6; fish, 2; miscellaneous, 3. Of these, meats, dairy products, poultry, eggs, and fish, making a total of 55 per cent, contain protein of high biological value; the other products, chiefly grains and their milled products, contain protein of relatively low biological value. This classification is based upon experiments with small animals in which each product was the only source of protein in the diet, and it does not necessarily indicate the nutritive value of the protein in these foods when consumed in a mixed diet. Since the protein in beef, as well as in many other animal products, greatly enhances the value of the protein in the grains, it is highly probable that the cereal proteins will be utilized very efficiently when they are included in a mixed diet containing meat or other animal proteins.

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THE NITROGEN COMPOUNDS OF THE RICE KERNEL AS COMPARED WITH THOSE OF OTHER CEREALS[1]

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INTRODUCTION

A more complete knowledge of the constituents of rice and of their significance is desirable, not only from a physiological and biochemical standpoint, but also from that of utility. After polypeptides and free amino acids had been shown to occur in the ungerminated kernel of wheat (*Triticum vulgare*) (10),[3] oats (*Avena sativa*) (8), corn (*Zea mays*) (9), and rye (*Secale cereale*) (11), it was reasonable to assume that those compounds were likely to be present also in the ungerminated kernel of rice (*Oryza sativa*). The fact that rice, however, is the chief food of more than half of the world's population and has maintained that position through thousands of years, coupled with the circumstance that polypeptides have never been reported to occur in the rice kernel, made it desirable to ascertain whether polypeptides and free amino acids occur in the different varieties of rice, and if so, in what proportions. The desire to clear up these points was augmented by the fact that ordinarily the results found in the literature are not related to definite varieties, and that different cereals have dissimilar characteristics. Thus, wheat is characterized by the high nitrogen and protein content of its kernel and is the only cereal the flour of which forms a coherent gluten (16). The rice kernel contains little nitrogen and protein, but it is distinguished by its high digestibility (14). In the corn kernel is included a considerable proportion of fat, and in the rye kernel a not inconsiderable quantity of a gummy substance (16). Other characteristics of these cereals will be mentioned later on.

REVIEW OF LITERATURE

The literature contains many records of analyses of the constituents of rice, a few of which will be reviewed. The analytical results of Wise and Broomell (24, p. 27) show that Honduras rice (fancy head, uncoated) contains 0.38 per cent ash, 0.19 per cent ether extract, 0.24 per cent crude fiber, 8.74 per cent protein, and 2.20 per cent pentosans, calculated to the moisture-free basis. Rosenheim and Kajiura (17) state that the proteins in table rice make up about 7 per cent of the grain as used for food. They class the proteins as rice globulin, rice albumin, and oryzenin. These results were confirmed by Suzuki, Yoshimura, and Fuji (23), who state that bran-free rice contains 1.200 per cent of total nitrogen, 1.165 per cent of protein nitrogen, and 0.035 per cent of nonprotein nitro-

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[1] Received for publication Sept. 2, 1926; issued April, 1927.
[2] Credit is due J. G. Wangler, formerly junior chemist of the Office of Plant Geography and Physiology, Bureau of Plant Industry, for assistance in doing part of the analytical work.
[3] Reference is made by number (italic) to "Literature cited," p. 324.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 4
Feb. 15, 1927,
Key No. G-561

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gen. Osborne, Van Slyke, Leavenworth, and Vinograd (14) have estimated the distribution of nitrogen among the hydrolytic products of oryzenin, the chief protein of the endosperm of rice, and have come to the conclusion that in its general amino-acid make-up it resembles the proteins of animal tissues in a higher degree than do the proteins of wheat or maize. This, in their opinion, explains the extensive use of rice as an exclusive diet throughout the Orient in spite of its low protein content.

From this reveiw it will be seen that these papers contain chiefly data as to the quantity of total nitrogen, protein nitrogen, and nonprotein nitrogen in the rice kernel; and these papers are fairly characteristic of the rest of the literature on rice. No information could be found in regard to the nature of the nonprotein nitrogen, to say nothing of polypeptides, which are not even mentioned in the literature.

RICE VARIETIES EMPLOYED

Four commercially known varieties of rice were used for the experiments recorded in this paper. Wataribune is a long-established commercial variety. It was grown for the first time in this country at Webster, Tex., in 1908, by a Japanese farmer who imported the seed from Japan. It is a rice of high-yielding capacity and is the principal variety cultivated in California. It belongs to the short-grain rices, which have small stalks, narrow leaves, and short kernels (*2, 12*).

Blue Rose is also a long-established commercial variety, the result of a selection made by Wright from an unknown variety found by Shoemaker in 1907 in a field of Japanese rice east of Jennings, La. Plants of this unknown variety were given by Shoemaker to Wright, who isolated a strain which was later offered for sale under the name Blue Rose. It is the leading variety of medium-grain rice and is grown chiefly in the Southern States (*2, 12, 15*).

Another long-known commercial variety is Honduras, which is a long-grain rice, imported from Honduras into Louisiana probably as early as 1890. It is probably a strain of the Creole variety, which is extensively raised in Morelos, Mexico. This rice is widely known and valued for its excellent cooking quality (*2, 15*).

Fortuna, which is a new variety, is a pure-line selection from the Pa Chiam variety, obtained in 1905 by the United States Department of Agriculture from the Department of Agriculture of Formosa. The selection was made by C. E. Chambliss and J. M. Jenkins in 1911 at the Rice Experiment Station, Crowley, La. (*2, 3*).

Only brown rice, i. e., seed rice, from which the husk had been removed, was used in the experiments here described. The rice samples were first carefully freed from weeds (red rice), seed rice, shriveled kernels, punctured rice (damaged by insects), chaff, and all foreign matter. They were then dried in an electric oven at about 55° C. for from two to three days, ground, passed through a 40-mesh sieve, and put into sealed jars ready for use.

METHODS AND RESULTS

Some general information with regard to the four rice varieties investigated will be found in Table 1. This investigation was begun in 1923, but owing to pressure of other work it had to be discontinued, and was resumed in January, 1926. For this reason

the data obtained from samples of the 1923 crop and from samples of the 1925 crop are given separately.

Table 1 shows that all the rice samples of 1925 have a lower moisture content than the corresponding samples of 1923. This may be due to the fact that 1925 was a drier season, or to the fact that the climatic conditions at the time of harvest in 1925 were drier than in 1923.

TABLE 1.—*Percentage of moisture, total nitrogen, protein nitrogen, nonprotein nitrogen, and ash in brown rice*

Year grown and variety	Where grown	Moisture in air-dried rice	Total nitrogen in oven-dried rice	Protein nitrogen in—		Nonprotein nitrogen in—		Ash in—	
				Oven-dried rice	Terms of total nitrogen of oven-dried rice	Oven-dried rice	Terms of total nitrogen of oven-dried rice	Air-dried rice	Oven-dried rice
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct*	*P. ct*
1923 Wataribune, C. I. 1561.	Sacramento Valley, Calif.	8.10	1.19					1.30	1.39
		7.93	1.19					1.29	1.39
		8.01	1.17						
		8.10							
Average		8.04	1.18					1.30	1.39
Blue Rose, C. I. 1962.	Stuttgart, Ark [a]	10.41	1.29					1.24	1.35
		10.27	1.29					1.25	1.36
		10.23	1.29						
		10.15	1.33						
Average		10.27	1.30					1.25	1.36
Blue Rose, C. I. 1962.	Rice Experiment Station, Crowley, La.	11.05	1.39					1.20	1.31
		10.96	1.38					1.19	1.30
		10.99	1.39						
		10.87							
Average		10.97	1.39					1.20	1.31
Honduras, C. I. 1643.	Rice Experiment Station, Crowley, La.	12.67	1.69					1.22	1.35
		12.49	1.71					1.23	1.36
			1.70						
Average		12.58	1.70					1.23	1.36
Fortuna, C. I 1344.	Rice Experiment Station, Cowley, La.	11.39	1.53					.88	.96
		11.24	1.53					.89	.97
		11.14	1.53						
			1.52						
Average		11.26	1.53					.89	.97
1925 Wataribune, C. I. 1561.	Rice Experiment Station, Crowley, La.		1.25	1.18	95.33	([b])	([b])	.86	.93
		8.00	1.24	1.18	95.33	0.062	5.02	.86	.93
		7.90	1.23						
Average		7.95	1.24	1 18	95.33	.062	5.02	.86	.93
Blue Rose, C. I. 1962.	Rice Experiment Station, Crowley, La.	7.44	1.54	1.46	96 92	.060	4.00	1.10	1.19
		7.25	1.51	1.43	94.92			1.11	1.19
			1.48						
Average		7.35	1.51	1.45	95.92	.060	4.00	1.11	1.19
Honduras, C. I. 1643.	Rice Experiment Station, Crowley, La.	8.07	1.38	1.34	97.12	.055	4.02	1.42	1.53
		8.07	1.40					1.45	1.55
			1.37						
Average		8.07	1.38	1.34	97.12	.055	4.02	1.44	1.54
Fortuna, C. I. 1344.	Rice Experiment Station, Crowley, La.	7.55	1.46	1.37	94.17	.093	6.40	1.01	1.09
		7.39	1.45	1.37	94.17	.095	6.52	1.01	1.09
			1.46						
Average		7.47	1.46	1.37	94.17	.094	6.46	1.01	1.09

[a] Grown by the Arkansas Rice Growers' Association.   [b] Lost.

Concerning total nitrogen, it will be seen that in 1923 Honduras had the highest percentage, 1.70; Fortuna the next highest, 1.53; Blue Rose from Louisiana, 1.39; Blue Rose from Arkansas, 1.30; and Wataribune, 1.18; while in 1925 Blue Rose from Louisiana had the most, 1.51; Fortuna had 1.46; Honduras, 1.38; and Wataribune, 1.24. It will also be noticed that Wataribune had practically the same percentage in both seasons, as did Fortuna also; that Honduras dropped from the highest place in 1923 to the third place in 1925, the reverse being the case with Blue Rose from Louisiana.

The quantity of protein nitrogen, calculated on the basis of the oven-dried rice, runs parallel with the quantity of total nitrogen. Thus, for instance, Blue Rose and Wataribune, having the highest and the lowest percentages of total nitrogen, respectively, in 1925, had also the highest and lowest percentages of protein nitrogen. As would be expected, the reverse was in general true of nonprotein nitrogen content.

As Table 1 shows, the ash content tended to be rather uniform. Of the three varieties grown at the Rice Experiment Station at Crowley, La., in 1923, Honduras had the highest ash content, Blue Rose the next highest, and Fortuna the lowest. The same relationship in regard to ash content held good for these three varieties in 1925. However, the Wataribune grown there that year had less ash than any of the three mentioned above, although when grown in California in 1923 it had more than any of them, even more than the Blue Rose grown at Stuttgart, Ark., by the Arkansas Rice Growers' Association, which had a considerably higher ash content than the Blue Rose grown in Louisiana in 1923.

NITROGEN EXTRACTED BY WATER

In order to gain an insight into the nature of the nitrogen compounds in the rice kernel, it is necessary to extract them in such a way that they will not undergo any changes. This is best done by treating the rice flour with boiling hot water, which destroys the enzymes normally present in plant tissues. Preliminary trials were made to ascertain just how much nitrogen is extracted by water. The procedure was as follows: Six flasks, each containing 25 gm. of rice flour, were treated with 500 c. c. of boiling hot ammonia-free water and kept on the water bath for 15 to 30 minutes, after which their contents were centrifuged. The solid residues were treated with boiling hot water once more. The combined extracts were then evaporated in a vacuum, the concentrated substance was centrifuged again, and the supernatant liquids were made up to 500 c. c. Two equal portions were oxidized according to the Kjeldahl method. The results obtained are recorded in Table 2.

An examination of Table 2 shows that water extracted more nitrogen from the Blue Rose variety than it did from the Wataribune rice, and still more from the Honduras variety. This was due partly to the fact that the percentages of total nitrogen in Blue Rose and Honduras are higher than the percentage in Wataribune.

TABLE 2.—*Percentage of nitrogen extracted by water from brown rice*

Sam- ple No.	Variety	Quantity of water extract used	Equivalent of extract in terms of—		Nitrogen extracted by water	
			Air-dry rice	Oven-dried rice	As percentage of oven-dried rice	As percentage of total nitrogen
		C. c	*Gm.*	*Gm.*		
1	Wataribune	200	60.0	55.40	0.031	2.43
2	___do___	200	60 0	55.40	.030	2 39
	Average				.031	2 41
3	Blue Rose	200	60.0	55.72	.055	3.64
4	___do___	200	60 0	55 72	055	3 63
	Average				055	3.64
5	Honduras	200	60.0	55.85	.056	4 08
6	___do___	200	60.0	55 85	057	4 14
	Average				.057	4 11

ACIDITY OF RICE EXTRACTS

In the course of the experimentation it was noticed that aqueous extracts of rice flour showed acid reaction. In order to ascertain the degree of acidity in the different varieties quantitatively, 25 c. c. portions of the aqueous extract, obtained in the manner outlined above, were diluted with 50 c. c. of distilled water, which was perfectly neutral to phenolphthalein. This dilution was at once titrated with tenth-normal sodium hydroxide, phenolphthalein being used as the indicator. The data are reported in Table 3.

TABLE 3.—*Acidity in rice of different varieties*

Sam- ple No.	Variety	Equivalent of extract used in terms of—		N/10 NaOH used for neutral- ization
		Air- dry rice	Oven- dried rice	
		Gm.	*Gm.*	*C. c.*
1	Wataribune	7.5	6.925	0.93
2	___do___	7.5	6.925	.93
3	___do___	7.5	6.965	.94
	Average			.93
4	Blue Rose	7.5	6.965	1.96
5	___do___	7.5	6.965	1.92
6	___do___	7.5	6.925	1.93
	Average			1.94
7	Honduras	7.5	6.981	3.12
8	___do___	7 5	6.981	3.02
9	___do___	7.5	6.981	3.07
	Average			3.07

By referring to Table 3 it will be seen that Honduras and Wataribune show the highest and lowest acidity, respectively, while Blu. Rose is between the two.

EXTRACTION OF THE VARIOUS NITROGEN COMPOUNDS

Since only about 5 per cent of the total nitrogen of rice is made up of nonnitrogenous compounds and only part of these is extracted by water, it seemed necessary to use a considerable quantity of rice in order to demonstrate the nature and quantity of the nitrogen compounds occurring in the rice kernel. Hence, several series of six flasks were provided, each flask containing 25 gm. of flour and treated with 500 c. c. of boiling hot water from which the ammonia had been removed by long boiling. The flasks were kept on the steam bath for about half an hour. After that their contents were partly filtered on Büchner filters with suction and partly centrifuged. The solid residues were combined and extracted once more in like manner. All of the extracts were then evaporated under reduced pressure, the substance thus concentrated was centrifuged again, and the combined supernatant liquids were finally evaporated to dryness in vacuo. The dry residue of the aqueous extract was then extracted with 85 per cent alcohol in order to get rid of impurities such as starch, proteins, and inorganic salts.

The alcoholic extract obtained was filtered and again evaporated to dryness under diminished pressure. The residual yellow sirup which resulted was dissolved in hot water, filtered, cooled, and made up to 100 c. c. in two 10 c. c. portions, of which the nitrogen was estimated according to the Kjeldahl method, while 10 c. c. was used for qualitative tests.

PHOSPHOTUNGSTIC ACID PRECIPITATE

The remaining 70 c. c. of the solution was made up to 100 c. c. or its multiple, depending on the amount of nitrogen present. To every 100 c. c. of the solution was added 5 gm. of concentrated sulphuric acid and ordinarily 30 c. c. of a phosphotungstic acid solution containing 20 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. After 24 hours the individual precipitates were filtered off on separate filters and washed with a solution containing 2.5 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. These were then oxidized according to the Kjeldahl method.

DETERMINATION OF ACID AMIDES

To the combined filtrates and washings from the various phosphotungstic precipitates calcium hydroxide was added to slight acidity, then barium hydroxide was added until distinct alkalinity was indicated. The whole was then saturated with carbon dioxide, boiled, and filtered on a Büchner filter. The cake remaining on the Büchner was extracted for the second time with boiling hot ammonia-free water. When the combined filtrates and washings, which were then concentrated in vacuo, were filtered, they left on the filter white crystals, identified as gypsum.

The filtrate from the gypsum crystals was made up to 200 c. c. In two 10 c. c. portions of this the nitrogen was estimated according to the Kjeldahl method. The 180 c. c. was made up to 200 c. c. and divided into two 100 c. c. portions. Each of these, on receiving 9 c. c. of concentrated hydrochloric acid, was boiled in a glycerin

bath for two hours, a reflux condenser being used.   On cooling, each hydrolysate was almost neutralized with sodium hydroxide and distilled with magnesium oxide previously reduced to cream with 100 c. c. of water.   The titration of the distillates then gave the ammoniacal nitrogen corresponding to the acid amides present.

HUMIN NITROGEN

The residues in the two Kjeldahl flasks after the ammonia had been driven off were decanted through two Büchners, each of which was provided with a hardened filter.   Each Kjeldahl flask was then treated a number of times with boiling hot ammonia-free water and the contents were decanted each time through the Büchners.   These were next washed with hot water and sucked perfectly dry.   The black substance could then be easily removed from the hardened filters and finally washed down with water quantitatively into 500 c. c. Kjeldahl flasks.   The contents of both the Kjeldahl flasks were oxidized in the usual way and distilled, a procedure which yielded the humin nitrogen.

NITROGEN OF AMINO ACIDS

The filtrates and washings from the two magnesium oxide residues were together concentrated on the water bath and made up to 200 c. c.   Of this solution 100 c. c. was used for the estimation of the amino nitrogen, while the other 100 c. c. was employed for the determination of the peptide nitrogen, on hydrolysis.   Of the first 100 c. c., two portions of 10 c. c. each were Kjeldahlized in order to ascertain the quantity of nitrogen present, 30 c. c. was used for qualitative tests, while in 50 c. c. the amino nitrogen was estimated by Sörensen's method (*6, 7, 21*).

PEPTIDE NITROGEN

The work of Fischer (*4, p. 23–53*) showed that the polypeptides represent complex compounds in which the amino acids are linked amidelike, and that a complete hydrolysis splits them so that free amino acids result.   Hence, the procedure in estimating peptide nitrogen was as follows: To the 100 c. c. of the solution supposed to contain amino acids and polypeptides, concentrated hydrochloric acid was added to a concentration of 20 per cent.   The solution was boiled in a glycerin bath under a reflux condenser for 12 hours (the temperature of the bath was about 125° to 127° C.).   The hydrolysate was then evaporated on the water bath practically to dryness, taken up with hot water, boiled for a few minutes with carbon black (if necessary), again concentrated on the water bath, and made up to 100 c. c. In two 10 c. c. portions of this the nitrogen was estimated according to the Kjeldahl method; in the 50 c. c. the total amino nitrogen was determined by the formol-titration method, and the residual solution was employed for qualitative tests.   Since the amino nitrogen found here represented not only the peptide nitrogen resulting from the hydrolysis but also the free amino nitrogen which was present as such in the solution, it was necessary to subtract this latter nitrogen from the total amino nitrogen found in order to obtain the peptide nitrogen only.   The results obtained by the methods outlined are summarized in Table 4.

TABLE 4.—*Distribution of the nonprotein nitrogen in the ungerminated rice kernel (brown rice)*

Variety and year grown	Nitrogen in phosphotungstic precipitate	Nitrogen of acid amides	Humin nitrogen	Nitrogen of amino acids	Peptide nitrogen
	Percentage of oven-dried rice				
Wataribune.					
1925	0.015	0.002	0.003	0.009	0.010
1923		.002	.003	.010	.011
Average	.015	002	.003	.010	.011
Blue Rose					
1925	.029	.002	.003	.021	.025
1923		.003	.003	.021	.024
Average	.029	.003	.003	.021	025
Honduras					
1925	.030	.003	.008	.014	.015
1923		.003	.007	.015	.017
Average	.030	.003	.008	.015	.016
	Percentage of total nitrogen of oven-dried rice				
Wataribune					
1925	1 22	0.14	0.21	0.72	0.82
1923		.14	.27	.77	.87
Average	1.22	.14	.24	.75	.85
Blue Rose					
1925	1.91	16	.23	1.38	1 64
1923		.17	.22	1.42	1 57
Average	1.91	17	.23	1.40	1.61
Honduras					
1925	2 15	.24	.56	.98	1.07
1923		.21	.52	1.06	1.23
Average	2.15	.23	54	1.02	1.15
	Percentage of water-soluble nitrogen of oven-dried rice				
Wataribune:					
1925	50.53	5.78	8 92	29.90	33.83
1923		5 98	11 28	31 96	36 25
Average	50.53	5.88	10.10	30.93	35.04
Blue Rose:					
1925	52.38	4.31	6.32	37.84	45.01
1923		4.54	6.01	38.93	43.20
Average	52 38	4.43	6.17	38.39	44.11
Honduras:					
1925	52.25	5.76	13.53	23 90	26.04
1923		5.19	12.58	25.88	29.84
Average	52 25	5.48	13 06	24 89	27.94

In Table 4 it will be noted that the differences in the nitrogen in the phosphotungstic precipitate of the several varieties are comparatively small. When calculated on the basis of the oven-dried kernel and on the basis of the total nitrogen of the oven-dried kernel, Wataribune and Honduras have, respectively, the lowest and highest percentage of nitrogen, while that in Blue Rose is between the two. The same relationship holds good for the nitrogen of acid amides, when calculated on the basis of the total nitrogen and of the water-soluble nitrogen. With regard to the percentage of humin nitrogen, it will be seen that it is highest in Honduras and lowest in Blue Rose, while that in Wataribune is between the two. The percentage of amino acid nitrogen is highest in Blue Rose (0.021 and 1.40); next in order come the percentages in Honduras (0.015 and 1.02) and Wataribune (0.010 and 0.75), calculated, respectively, on the oven-dried kernel and on the total nitrogen of the oven-dried kernel. The percentage of peptide nitrogen is highest in Blue Rose (0.025 and 1.61), next highest in Honduras (0.016 and 1.15), and lowest in Wataribune (0.011 and 0.85), calculated to the oven-dried kernel and to the total nitrogen of the oven-dried kernel.

GENERAL DISCUSSION AND INTERPRETATION OF RESULTS

TOTAL NITROGEN IN THE VARIOUS CEREALS

Table 5 needs some explanation. In order readily to compare the total nitrogen in different varieties, it was deemed best to consider the amount of nitrogen in the variety of each cereal richest in nitrogen as 100. It is to this basis that the amounts of nitrogen in the other varieties were calculated. Further, in order to have a broad general basis for comparing the total nitrogen in all of the cereals studied, the highest percentage of nitrogen, which was found to occur in Marquis (3.04 per cent), calculated on the oven-dried wheat kernel, was considered (in column 6) as 100, and the nitrogen of the other cereals was compared with it.

TABLE 5.—*Proportion of total nitrogen in the ungerminated kernel of various cereals*

Sample No.	Kind and variety	Where and when grown	Total nitrogen calculated on the basis of—		
			Oven-dried cereal	Variety richest in nitrogen within each cereal considered as 100	Cereal richest in nitrogen considered as 100
	Wheat		*Per cent*	*Per cent*	*Per cent*
1	Fultz, C. I. 3598	Arlington Farm, Rosslyn, Va., 1920	1.80	59.21	59 21
2	Kanred, C. I. 5146	Hays Branch Experiment Station, Hays, Kans., 1922.	2 83	93.09	93.09
3	Kubanka, C. I.	1920 [a]	3.03	99.67	99.67
4	Marquis, C. I. 3641	Dickinson Substation, Dickinson, N. Dak., 1920.	3 04	100 00	100.00
	Oats				
5	Swedish Select, C. I. 134	Dickinson, N Dak., 1920	2.59	100.00	85.20
6	Iowar, C. I. 847	Iowa Agriculture Experiment Station, Ames, Iowa, 1922.	2.40	92.66	78.95
7	Victory, C. I. 560	Dickinson, N. Dak., 1922	2.02	77.99	66.45
8	Winter Turf, C. I. 435–4	Arlington Farm, Rosslyn, Va., 1922	1.50	57.91	49.34
	Corn				
9	Four County	Iowa Agriculture Experiment Station, Ames, Iowa, 1921.	1.70	100.00	55.92
10	United States Selection No. 77.	Piketon, Ohio, 1919	1.59	93.53	52.30
11	Hall Gold Nugget Selection No. 193.	Rhineback, N. Y., 1921	1.46	85.88	48.03
	Rye				
12	North Dakota No. 959	Dickinson, N. Dak., 1922	2.41	100.00	79.28
13	Von Rümker, C. I. 133	1923 [a]	1.84	76 35	60.53
14	Reg. Rosen No. R 22198	Parma, Mich., 1922	1.65	68 46	54.28
	Rice				
15	Wataribune, C. I. 1561	Sacramento Valley, Calif , 1923	1.18	69 41	38.82
16	Blue Rose, C. I. 1962	Stuttgart, Ark , 1923	1.30	76.47	42.76
17	Blue Rose, C. I. 1962	Rice Experiment Station, Crowley, La., 1923.	1.39	81.76	45.72
18	Honduras, C. I. 1643	do	1.70	100.00	55.92
19	Fortuna, C. I. 1344	do	1 53	90.00	50.33
20	Wataribune, C. I. 1561	Rice Experiment Station, Crowley, La., 1925.	1 24	72.94	40.79
21	Blue Rose, C. I. 1962	do	1 51	88 82	49 67
22	Honduras, C. I. 1643	do	1.38	81 18	45.39
23	Fortuna, C. I. 1344	do	1.46	85.88	48 03

[a] The place where grown could not be ascertained.

Inspection of column 5 of Table 5 shows marked differences in the total nitrogen of the several varieties of each cereal. Thus, of the corn varieties studied, Four County corn has the highest nitrogen content (100 per cent), and United States Selection No. 77 (93.53 per cent) and Hall Gold Nugget Selection No. 193 (85.88 per cent) rank next in the order named. On the other hand, when the various cereals are compared (column 6) it will be found that there are characteristic differences among them. Thus, the wheat and rice kernels have, respectively, the highest and lowest nitrogen content, and between the two lie the percentages of nitrogen in oat, rye, and corn kernels, which decrease in the order named. These differences in the nitrogen content of the various cereals may be due, aside from inheritance, to a variety of causes, such as water and starch content of the kernels, quality of the seed, soil and climatic conditions, and last but not least, the method used for the nitrogen determination. It is evident that, other considerations being equal, the kernels should be the poorer in nitrogen the more water and starch they contain, because in this case they may be spoken of as kernels with a "dilute" content as far as the protein nitrogen is concerned. On the other hand, the kernels should be richer in nitrogen the more nitrogen there is available in the soil producing them, the higher the quality of the seed, and the more favorable the climatic conditions. Inasmuch as the total nitrogen in all the cereals investigated was calculated on the basis of the oven-dried kernel and the method employed for the nitrogen estimation was practically identical throughout these investigations, the water content and method do not play here any special rôle. The starch content will not be considered here because its determination was not contemplated in our work, nor will the quality of the various seeds as to which no information was obtained be taken into consideration. As to the powerful influences of soil and climate, they can best be illustrated by a comparison of the Blue Rose rices grown at Stuttgart, Ark., in 1923 (No. 16) and at Crowley, La., in 1923 (No. 17), with that grown at Crowley, La., in 1925 (No. 21). They had, respectively, 76.47, 81.76, and 88.82 per cent of nitrogen, considering the quantity of nitrogen in 1923 as 100. Likewise, Honduras (No. 18) and Fortuna (No. 19) from Crowley, La., the nitrogen in which in 1923 was assigned the comparative values of 100 and 90 per cent, respectively, had in 1925, on the same basis, but 81.18 (No. 22) and 85.88 (No. 23) per cent, respectively.

PROTEIN NITROGEN AND NONPROTEIN NITROGEN IN THE VARIOUS CEREALS

Table 6 shows that protein nitrogen makes up the great bulk of the total nitrogen, and ranges, on an average, from about 87, 88, and 90 per cent in rye, wheat, and oats, respectively, to more than 95 per cent in corn and rice. While the proportion of protein nitrogen is smaller in rye, wheat, and oats than in corn and rice, the absolute quantity of protein nitrogen, and hence of proteins, is considerably larger in the first three cereals mentioned because of their higher percentage of total nitrogen than it is in the latter two cereals. Inasmuch as the total nitrogen in the kernel of the various cereals is, aside from inheritance, attributable more or less to factors such as soil, climate, and quality of seed, it seems not unreasonable to ascribe to the same causes also the greater or smaller proportion of protein nitrogen in the various kernels.

TABLE 6.—*Proportion of protein and of nonprotein nitrogen in the ungerminated kernel of the various cereals*

Sample No.	Cereal and variety	Where and when grown	Protein nitrogen, calculated on the basis of—		Nonprotein nitrogen, calculated on the basis of—	
			Oven-dried kernel	Total nitrogen of oven-dried kernel	Oven-dried kernel	Total nitrogen of oven-dried kernel
	Wheat		*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Fultz, C. I 3598	Arlington Farm, Rosslyn, Va., 1920.	1 64	91 76	0.15	8.24
2	Kanred, C. I. 5146	Hays Branch Experiment Station, Hays, Kans., 1922.	2.47	87.01	.37	12.99
3	Kubanka, C. I	1920 *a*	2.73	86.73	.40	13.27
4	Marquis, C I. 3641	Dickinson Substation, Dickinson, N. Dak., 1920.	2.65	87.26	.39	12.74
	Oats					
5	Swedish Select, C. I. 134	Dickinson, N. Dak., 1920	2.30	88.65	.29	11.35
6	Iowar, C. I 847	Iowa Agricultural Experiment Station, Ames, Iowa, 1922	2.17	90.52	.23	9.48
7	Victory, C. I. 560	Dickinson, N Dak , 1922	1 82	90 21	.20	9 79
8	Winter Turf, C.I. 435-4.	Arlington Farm, Rosslyn, Va., 1922.	1.36	91.03	.14	8.97
	Corn					
9	Four County	Iowa Agricultural Experiment Station, Ames, Iowa, 1921.	1.63	95.70	.073	4 30
10	United States Selection No. 77.	Piketon, Ohio, 1919	1.52	95 69	.068	4.31
11	Hall Gold Nugget Selection No. 193.	Rhinebeck, N Y., 1921	1.39	95.59	.064	4.41
	Rye					
12	North Dakota No. 959.	Dickinson, N. Dak., 1922	2.02	83.89	.39	16.11
13	Von Rumker, C. I. 133.	1923 *a*	1.58	85.73	.26	14.27
14	Reg. Rosen, No. R 22198.	Parma, Mich , 1922	1.51	91 52	.14	8.48
	Rice					
15	Wataribune, C. I. 1561.	Rice Experiment Station, Crowley, La , 1925.	1 18	95 33	.062	5.02
16	Blue Rose, C. I. 1962	do	1 45	95.92	.060	4 00
17	Honduras, C. I 1643	do	1 34	97.12	.055	4.02
18	Fortuna, C. I. 1344	do	1.37	94.17	.094	6.46

a The place where grown could not be ascertained

There is, however, one factor which may have considerably more influence upon the proportion of protein nitrogen in the cereal kernel than the conditions mentioned above, namely, the greater or lesser ripeness of the seed. From observations of Schulze (*19, 20*) and other investigators the conclusion seems to be justified that the proportion of proteins and nonproteins is more or less fluctuating, depending on the state of ripeness of the seed. Unripe seed ordinarily contains a higher percentage of nonproteins and ripe seed ordinarily contains a higher percentage of proteins, and vice versa. Unfortunately, there is no information as to the state of ripeness at the time of harvest of any of the varieties investigated.

That the nonprotein nitrogen, which was either directly estimated in the filtrate from the protein nitrogen according to Stutzer's method (*22*), or calculated by difference from 100, would stand in reverse ratio to the protein nitrogen, was to be expected.

ACID AMIDE NITROGEN AND HUMIN NITROGEN IN THE VARIOUS CEREALS

In Table 7 it is seen that in general rye and rice have the highest and lowest percentages of acid amide nitrogen, respectively, the intermediate cereals, wheat, corn, and oats, showing, in the order named, a gradual decrease in percentage of acid amide nitrogen.

Yet the absolute quantity of the nitrogen corresponding to acid amides is very small. As will be remembered, the total nonprotein nitrogen in the various cereals (Table 6) ranges, in round figures, from about 4 or 5 per cent in corn and rice to about 10, 12, and 13 per cent in oats, wheat, and rye, respectively, calculated on the basis of the total nitrogen. Of this small percentage of nonprotein nitrogen, however, only part can be extracted with water, which part is distributed among the various nitrogenous compounds, such as acid amides, amino acids, and polypeptides. Nevertheless, their presence in the ungerminated kernel of the cereals seems to be physiologically important, as will subsequently be seen.

TABLE 7.—*Percentage of acid amide and humin nitrogen in the ungerminated kernel of the various cereals*

Sample No.	Cereal and variety	Where and when grown	Acid amide nitrogen, calculated on the basis of—			Humin nitrogen, calculated on the basis of—			Remarks
			Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel	Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel	
	Wheat		*P. ct.*	*P. ct.*	*P. ct*	*P ct.*	*P. ct.*	*P. ct.*	
1	Fultz, C. I. 3598	Arlington Farm, Rossyln, Va., 1920.	0. 026	1. 46	8. 76				Cold-water extract.
2	Kanred, C. I. 5146.	Hays Branch Experiment Station, Hays, Kans., 1922.	. 053	1. 88	12. 99				Do.
3	Kubanka, C. I.	1920 [a]	. 052	1 72	12 61				Do.
4	Marquis, C. I. 3641.	Dickinson substation, Dickinson, N. Dak., 1920.	. 058	1. 91	12. 33				Do.
	Oats								
5	Swedish Select, C. I. 134.	Dickinson, N. Dak., 1920.	. 051	1. 95	13. 69				Do.
6	Iowar, C. I. 847	Iowa Agricultural Experiment Station, Ames, Iowa, 1922.	. 051	2 13	13. 45				Hot - water extract.
			. 046	1. 93	12. 18				Cold-water extract.
7	Victory, C. I. 560.	Dickinson, N. Dak., 1922.	. 032	1. 61	12. 03				Hot - water extract.
			. 029	1. 45	10. 86				Cold-water extract.
8	Winter turf, C. I. 435–4.	Arlington Farm, Rosslyn, Va .1922.	. 027	1. 80	15. 01				Do
	Corn								
9	Four County	Iowa Agricultural Experiment Station, Ames, Iowa, 1921.	. 034	2. 00	16. 58				Hot - water extract.
10	United States Selection No. 77.	Piketon, Ohio, 1919	. 018	1. 13	11. 64				Cold-water extract.
11	Hall Gold Nugget Selection No. 193.	Rhinebeck, N. Y., 1921.	. 021	1. 44	11. 15				Hot -water extract.
			. 022	1. 51	11. 32				Cold-water extract.
	Rye								
12	North Dakota No. 959.	Dickinson, N. Dak., 1922.	. 090	3 72	16. 32				Hot -water extract.
13	Von Rumker, C. I. 133.	1923 [a]	. 093	4. 96	18. 50				Do.
14	Reg Rosen, No. R. 22198.	Parma, Mich., 1922	. 069	4. 14	14. 28				Do.
	Rice								
15	Wataribune, C. I. 1561.	Rice Experiment Station, Crowley, La., 1925.	. 002	. 14	5. 88	0 003	0. 24	10. 10	Do.
16	Blue Rose, C. I. 1962.	do	. 003	. 17	4. 43	. 003	. 23	6. 17	Do.
17	Honduras, C. I. 1643.	do	. 003	. 23	5. 48	. 008	. 54	13. 06	Do.

[a] The place where grown could not be ascertained.

Table 7 shows that Honduras and Blue Rose have the highest and lowest proportion of humin nitrogen, respectively, the proportion in Wataribune being between the two. The absolute quantity of humin nitrogen, too, is very small, as has already been pointed out. The formation of humin bodies is chiefly, if not wholly, due to acid hydrolysis incidental to the estimation of acid amides (and polypeptides), and is to be ascribed to partial decomposition of tryptophan, tyrosine, cystine, and diamino acids present in plant and other biological materials, as was pointed out by Gortner and Holm (*5*) as well as by Roxas (*18*).

AMINO NITROGEN AND PEPTIDE NITROGEN IN THE VARIOUS CEREALS

By referring to Table 8 it will be noted that rice and rye have, respectively, the lowest and highest proportions of amino nitrogen, and that between them are wheat, oats, and corn, in which, in the order named, the proportion of amino nitrogen, calculated on the basis of the oven-dried kernel and of the total nitrogen of the oven-dried kernel, gradually increases. It will also be found that rice and rye have the lowest and highest percentage of peptide nitrogen, respectively, but that between the two are corn, oats, and wheat, in which, in the order named, the peptide nitrogen, calculated to the oven-dried kernel and to the total nitrogen of the oven-dried kernel, increases.

While the absolute quantity of amino and of peptide nitrogen is very small for the reasons outlined in connection with the acid amide and humin nitrogen, they all appear to be of considerable physiological importance. During the first two or three days of seed germination some soluble, diffusible, and readily transportable material is needed for building up the tissues of the young plantlet. The nonproteins contained in the seed represent such material. As soon, however, as chlorophyll appears and the enzymes are coming into play the plantlet is, of course, able to synthesize carbohydrates and to split the stored-up seed proteins, which are mostly insoluble and at any rate not diffusible, into smaller molecules (amino acids, acid amides, etc.), which can then be readily transported to the growing parts of the seedlings. The polypeptides, standing as they do between the amino acids on the one hand and the proteins on the other, may be split by the enzymes present into amino acids for translocation to points where they are needed, or they may represent the immediate material out of which the great protein structures are synthesized. From the standpoint of nutrition, the amino acids play an important rôle, since it has been shown by recent investigations of Osborne and Mendel (*13*) and Abderhalden (*1*) that the animal organism can build up proteins out of amino acids.

TABLE 8.—*Percentage of amino and peptide nitrogen in the ungerminated kernel of the various cereals*

Sample No.	Cereal and variety	Where and when grown	Amino nitrogen, calculated on the basis of—			Peptide nitrogen, calculated on the basis of—		
			Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel	Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel
	Wheat		*P. ct.*	*P. ct.*	*P. ct.*	*P ct.*	*P. ct.*	*P. ct.*
1	Fultz, C. I. 3598	Arlington Farm, Rosslyn, Va., 1920.	0.033	1 85	11 13	0.084	4.67	a 28 09
			029	1 61	9.72			
			.033	1.85	11.12			
2	Kanred, C I. 5146	Hays Branch Experiment Station, Hays, Kans, 1922.	.066	2.32	16 04	.111	3.89	a 26.86
			.067	2 38	16.46			
3	Kubanka, C. I.	1920 c	.047	1.55	11.41	.155	3.13	a 37.76
			.037	1.21	8 92			
			.039	1 28	9.41			
4	Marquis, C. I. 3641	Dickinson Substation, Dickinson, N. Dak , 1920.	.048	1.56	10.09	.151	4.98	a 32.20
			.060	1 95	12.63			
			.055	1.81	11.67			
	Oats							
5	Swedish Select, C. I. 134.	Dickinson, N. Dak., 1920	.064	2.48	17.39	.106	4.10	a 28.71
6	Iowar, C. I. 847	Iowa Agricultural Experiment Station, Ames, Iowa, 1922	.057	2.35	14.87	.098	4 07	b 25.69
						.084	3.49	a 22.03
7	Victory, C. I. 560	Dickinson, N. Dak., 1922	.040	2.02	15.08	.077	3.82	b 28.58
8	Winter Turf, C. I. 435-4.	Arlington Farm, Rosslyn, Va., 1922.	.025	1.65	13.79	.073	3.63	a 27.16
						.032	2.15	a 17.88
	Corn							
9	Four County	Iowa Agricultural Experiment Station, Ames, Iowa, 1921.	.044	2.59	21.68	.070	4.12	b 34.36
10	United States Selection No 77.	Piketon, Ohio, 1919	.042	2.64	28.11	.052	3.27	a 34.11
11	Hall Gold Nugget Selection No. 193.	Rhinebeck, N. Y., 1921	.052	3.56	27.57	.035	2.40	b 18.55
			.050	3.43	26.51	.039	2.67	a 20.66
	Rye							
12	North Dakota No. 959	Dickinson, N. Dak., 1922	.075	3.09	13.56	.162	6 69	b 29.36
13	Von Rümker, C I.133.	1923 a	101	5 39	20 10	.155	8.30	b 30 96
14	Reg Rosen No. R, 22198.	Parma, Mich., 1922	099	5.97	20.62	.070	4.21	h 14 55
	Rice							
15	Wataribune, C. I. 1561	Rice Experiment Station, Crowley. La., 1925.	.010	75	30.93	.011	.85	b 35 04
16	Blue Rose, C. I 1962	do	021	1 40	38.39	025	1 61	b 44.11
17	Honduras, C. I. 1643	do	.015	1.02	24.89	016	1.15	b 27 94

a Cold-water extract.　　b Hot-water extract.　　c The place where grown could not be ascertained.

BASIC NITROGEN AND THE ASH IN THE VARIOUS CEREALS

In Table 9, with "basic nitrogen" is included all the nitrogen obtained in the phosphotungstic precipitate, which may contain not only ammonia and the hexon bases (histidine, arginine, lysine), but also proteins, proteoses, and peptones. Table 9 shows that oats and rice have, respectively, the highest and lowest proportion of basic nitrogen, calculated on the basis of the oven-dried kernel and of the total nitrogen of the oven-dried kernel, the proportion in corn being between the two. It is worthy of note that the basic nitrogen makes up a considerable proportion of the water-soluble nitrogen, being greater than the percentage of any of the nonprotein nitrogenous

compounds, such as amino acids, acid amides, etc. With regard to the ash, it will be seen that oats has the highest percentage of ash, followed by the percentages in rye, corn, and rice in the order named.

TABLE 9.—*Percentage of basic nitrogen and of ash in the ungerminated kernel of the various cereals*

Sample No.	Cereal and variety	Where and when grown	Basic nitrogen, calculated on the basis of—			Ash, calculated on the basis of —	
			Oven-dried kernel	Total nitrogen of oven-dried kernel	Water-soluble nitrogen of oven-dried kernel	Air-dried kernel	Oven-dried kernel
	Oats		*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1	Swedish select, C. I. 134	Dickinson, N. Dak., 1920	0.165	6.40			3.25
2	Iowar, C. I. 847	Iowa Agricultural Experiment Station, Ames, Iowa, 1922.					3.39
3	Victory, C. I. 560	Dickinson, N. Dak., 1922	.111	5.48			3.15
4	Winter Turf, C. I. 435-4	Arlington Farm, Rossyln, Va., 1922.	.078	5.21			3.72
	Corn						
5	Four County	Iowa Agricultural Experiment Station, Ames, Iowa, 1921.	.076	4.47	37.35		1.40
6	United States Selection No. 77.	Piketon, Ohio, 1919	.061	3.84	40.11		1.33
7	Hall Gold Nugget Selection No. 193.	Rhinebeck, N. Y., 1921	.076	5.21	39.57		1.33
	Rye						
8	North Dakota, No. 959	Dickinson, N. Dak., 1922					1.79
9	Von Rümker, C. I. 133	1923 [a]					2.11
10	Reg. Rosen, No. R 22198	Parma, Mich., 1922					2.04
	Rice						
11	Wataribune, C. I. 1561	Sacramento Valley, Calif., 1923.				1.30	1.39
12	Blue Rose, C. I. 1962	Stuttgart, Ark., 1923				1.25	1.36
13	do	Rice Experiment Station, Crowley, La., 1923.				1.20	1.31
14	Honduras, C. I. 1643	do				1.23	1.36
15	Fortuna, C. I. 1344	do				0.89	0.97
16	Wataribune, C. I. 1561	Rice Experiment Station, Crowley, La., 1925.	.015	1.22	50.53	.86	.93
17	Blue Rose, C. I. 1962	do	.029	1.91	52.38	1.11	1.19
18	Honduras, C. I. 1643	do	.030	2.15	52.25	1.44	1.54
19	Fortuna, C. I. 1344	do				1.01	1.09

[a] The place where grown could not be ascertained.

Owing to their great complexity, the quantitative separation of the various compounds in cereals involves a number of difficulties. For this reason the data here presented can not be considered as perfectly quantitative. Yet when due allowance is made for unavoidable errors in such work, it is nevertheless true that if all the conditions and manipulations have been strictly identical throughout the investigation, as was the endeavor in this instance, the results are decidedly comparable.

SUMMARY

Of the rice varieties of the crop of 1923 studied, Honduras was characterized by the highest percentage of total nitrogen, 1.70. Fortuna had the next highest, 1.53, while the other varieties had

smaller percentages, Blue Rose from Louisiana, 1.39, Blue Rose from Arkansas, 1.30, and Wataribune, 1.18. Of the varieties of the 1925 crop studied, Blue Rose from Louisiana had the highest percentage of nitrogen, 1.51, Fortuna having 1.46, Honduras 1.38, and Wataribune 1.24.

It has been shown in this paper that polypeptides and free amino acids occur in the ungerminated rice kernel (brown rice).

The proportions of amino nitrogen in the varieties Wataribune, Blue Rose, and Honduras (all from Louisiana, crop of 1925) were, respectively, 0.010, 0.021, and 0.015 per cent, calculated on the oven-dried kernel, and 0.75, 1.40, and 1.02 per cent, calculated on the basis of the total nitrogen of the oven-dried kernel.

The acid amide nitrogen in the varieties Wataribune, Blue Rose, and Honduras (crop of 1925) constituted, respectively, 0.14, 0.17, and 0.23 per cent of the total nitrogen of the oven-dried kernel.

The nitrogen of polypeptides in the varieties Wataribune, Blue Rose, and Honduras (crop of 1925) made up, respectively, 0.011, 0.025, and 0.016 per cent of the oven-dried kernel, and 0.85, 1.61, and 1.15 per cent of the total nitrogen of the oven-dried kernel.

Considering the percentage of total nitrogen in Honduras (1.70), crop of 1923, as 100, it was found that Wataribune from California, Blue Rose from Arkansas, Blue Rose from Louisiana, and Fortuna— all of the 1923 crop—had, respectively, 69.41, 76.47, 81.76, and 90.00 per cent of nitrogen, while for the 1925 crop Wataribune from Louisiana and Honduras, Fortuna, and Blue Rose from Louisiana had, respectively, 72.94, 81.18, 85.88, and 88.82 per cent of nitrogen.

A comparison of the nitrogen compounds occurring in rice with those in wheat, rye, oats, and corn is given and their significance pointed out.

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PERIDERMIUM KURILENSE DIET. ON PINUS PUMILA PALL., AND PERIDERMIUM INDICUM N. SP. ON PINUS EXCELSA WALL.[1]

By Reginald H. Colley, *Pathologist,* and Minnie W. Taylor, *formerly Junior Pathologist, Office of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

There appear to be only three species of Peridermium which attack the stems of five-needled or white pines: *Cronartium ribicola* Fischer *(Peridermium strobi* Kleb.) on *Pinus albicaulis* Engelmann, *P. aristata* Engelmann, *P. ayacahuite* Ehrenb., *P. balfouriana* Balfour, *P. cembra* L., *P. excelsa* Wall., *P. flexilis* James, *P. koraiensis* Sieb and Zucc., *P. lambertiana* Douglas, *P. monticola* Douglas, *P. parviflora* Sieb and Zucc., *P. peuce* Gris., *P. strobiformis* Engelmann, and *P. strobus* L. *(5, 6)* [2] [3]; *Peridermium kurilense* Diet. *(4)* on *Pinus pumila* Pall.; and *Peridermium indicum* n. sp. on *Pinus excelsa.* The life history, morphology, and parasitism of *Cronartium ribicola* are now fairly well known. Either or both of the other two species, *Peridermium kurilense* and *P. indicum,* might become serious parasites if introduced into this country. At any rate their invasion would certainly complicate the problem of differential diagnosis in the western part of the United States, where *Cronartium ribicola* and *C. occidentale,* the piñon pine rust, are already present.

SOURCE OF MATERIAL

The material of *Peridermium kurilense* available for study consisted of one small piece of bark measuring approximately $17 \times 10 \times 3$ mm., bearing a few broken aecia. The specimen is No. 19047 in the herbarium of J. R. Weir. It is labelled as type material.

Two specimens of the species which is here named *Peridermium indicum* were lent for examination through the courtesy of E. J. Butler and W. McRae. The first specimen was sent by Butler to W. Stuart Moir, in 1921, and subsequently the writers had an opportunity to see it. The second specimen, sent directly from Pusa, India, by McRae, consisted of a single piece of twig about 2½ inches long and one-quarter of an inch in diameter, bearing 14 aecia. Both specimens were part of the same collection. The label with the second specimen reads, *"Peridermium complanatum* Barc. on *Pinus excelsa.* Kulu, N. W. Himalaya. 3–6–14 (June 3, 1914). Coll. R. S. Troup."* However, Barclay's original description *(1)* of *Aecidium complanatum* refers definitely to an aecium on the needles of *Pinus longifolia* Roxb.; and the name is therefore not applicable to the stem *Peridermium* on *Pinus excelsa.*

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 4
Feb. 15, 1927
Key No. G-569

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METHODS AND MEASUREMENTS

The aeciospores were mounted for study in the glycerin and glycerin-jelly mounting media used for mounting the urediniospores and aeciospores of *Cronartium ribicola* and *C. occidentale* (*2, 3*). All measurements were made with a filar micrometer. The thickness of wall reported refers to the thickest part of the side wall of the spores. The peridial cells were studied in sectional view. The wall thickness of the peridial cells refers to the wall thickness of the
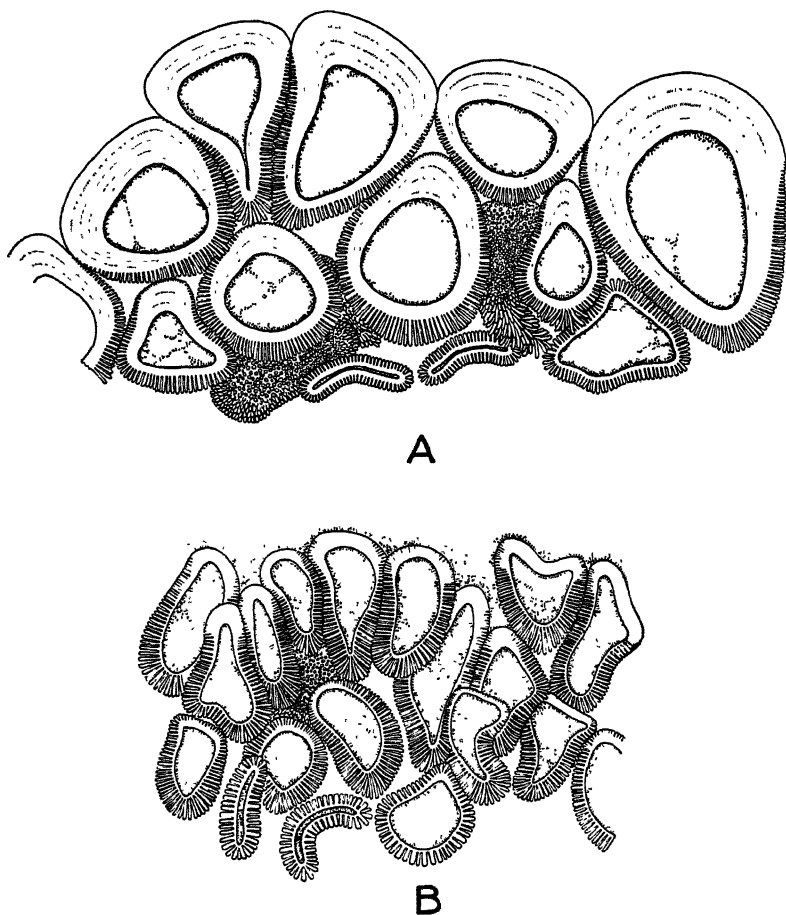
FIG. 1.—Sections through the peridia in the region near the dome: A, *Peridermium kurilense*; B, *Peridermium indicum*. × 625

outer or outside wall of the outer layer of cells in the peridium. The results of the measurement study are presented in Table 1. For the sake of comparison, data on species means of *C. ribicola* and *C. occidentale* (*3*) are also included.

Sectional views of the peridial cells made from material which appeared to be normal for the specimens are shown in Figure 1, A and B. The drawings do more to bring out the differences between the two species than any amount of description; and a comparison of the illustrations with others representing similar sections

from the peridia of *Cronartium ribicola* and *C. occidentale* (*3*) yields convincing evidence of the value of peridial-cell characters in diagnosis.

TABLE 1.—*Data on the size of the aeciospores, aeciospore tubercles, and peridial cells of Peridermium kurilense, P. indicum, Cronartium ribicola, and C. occidentale*

Aeciospores, aeciospore tubercles, and peridial cells	Basis	Length		Width		Wall		Ratio Mean length / Mean width
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Aeciospores.		μ		μ		μ		
Peridermium kurilense	200	27.3	3.5	21.4	2.3	4.24	0.89	1.27
Peridermium indicum	200	27.0	3.4	20.3	2 3	4.33	93	1.33
Cronartium ribicola	(a)	24.2	2 26	18.3	1.66	3.41	--------	1.32
Cronartium occidentale	(a)	26.8	2.79	19.0	2.12	3 85	--------	1.41
Aeciospore tubercles, end view.								
Peridermium kurilense	100	1.43	.31	1.05	.23	--------	--------	1.36
Peridermium indicum	100	1 41	.28	1.05	.23	--------	--------	1.34
Cronartium ribicola	(a)	1.52	.31	1.10	.21	--------	--------	1.38
Cronartium occidentale	(a)	2.29	.64	1.33	.27	--------	--------	1.72
Peridial cells:								
Peridermium kurilense	200	53.3	8.0	38.3	7.7	13.77	2.98	1.39
Peridermium indicum	200	38.4	5.9	24.9	4.6	3.69	.91	1.54
Cronartium ribicola	(a)	41.4	5.5	28.5	5.3	7.16	1.36	1.45
Cronartium occidentale	(a)	27.4	4.1	19.1	3 5	4 68	.89	1.43

[a] Species means based on numerous specimens. For detailed data see (*3*).

DESCRIPTION

The following descriptions combine the biometric and morphologic data:

Peridermium kurilense Diet.

Aecia usually discrete, occasionally confluent.

Peridia apparently persistent, 2 to 3, occasionally 4, cells thick; outer layer of cells in region near dome, sectional view, long and short dimensions (basis 200 measurements from 1 specimen) $53.3 \times 38.3\mu$, standard range [4] $45.4 - 61.3 \times 30.6 - 46.0\mu$, ratio mean length divided by mean width 1.39; outer wall of cells in outer layer smooth, 13.77μ thick, generally somewhat thicker than inner wall of the same cells; walls striated; inner wall marked with thin tubercles which are 5 to 10 times as long as they are broad; walls of cells in the second layer marked with tubercles over their entire surface, the tubercles, however, being very short on the ends of the cells toward the outside of the peridium and full length on the ends of the cells toward the inside of the peridium; outer walls of the cells in the second layer often thicker than the inner walls of the same cell; walls of cells in third layer generally uniform in thickness, marked over entire surface with uniformly distributed tubercles.

Aeciospores obovoid to ellipsoid, sometimes subspherical, generally smoothly curved in outline, (basis 200 spores) $27.3 \times 21.4\mu$; standard range $23.8 - 30.8 \times 19.1 - 23.7\mu$; ratio mean length divided by mean width 1.27; wall 4.24μ thick, standard range $3.35 - 5.13\mu$; wall partly smooth and partly marked with tubercles. the smooth area fissured near junction with tubercles; tubercles fairly regular in outline in view, (basis 100 measurements) $1.43 \times 1.05\mu$, standard range $1.12 - 1.47 \times 0.82 - 1.28\mu$; ratio mean length divided by mean width 1.36.

On twigs of *Pinus pumila* Pall. (=*P. cembra* var. *pumila* Pall.), Kurile Island, Japan. Specimen No. 19047 Herb. J. R. Weir.

Peridermium indicum n. sp.

Aecia usually discrete.

Peridia persistent, 3 to 4 cells thick; outer layer of cell in region near dome, sectional view, long and short dimensions (basis 200 measurements) $38.4 \times 24.9\mu$,

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[4] The lower and upper limits of the standard range differ from the mean by the amount of the standard deviation.

standard range 32 5 — 44.3 × 20.3 — 29.5μ, ratio mean length divided by mean width 1.54; outer wall of cells in outer layer smooth, 3.69μ thick, generally somewhat thinner than the inner wall plus its tubercles; outer wall sometimes fissured but bearing no tubercles; inner wall marked with thin tubercles which are five to ten times as long as they are broad; walls of the second and third layers of cells marked essentially the same as the cells in the outer layer, except that the walls on the outer ends of the cells are sometimes studded with short tubercles; walls of cells in the inner layer nearly uniform in thickness, marked with uniformly distributed tubercles.

Aeciospores obovoid to ellipsoid, generally smoothly curved in outline, (basis 200 spores) 27.0 × 20.3μ, standard range 23.6 — 30.4 × 18.0 — 22.6μ; ratio mean length divided by mean width 1.33; wall 4.33μ thick, standard range 3.40 — 5.26μ; wall partly smooth and partly marked with tubercles, the smooth area fissured near junction with tubercles; tubercles fairly regular in outline in end view, (basis 100 measurements) 1.41 × 1.05μ, standard range 1.13 — 1.69 × 0.82 — 1.28μ; ratio mean length divided by mean width 1.34.

On twigs and branches of *Pinus excelsa* Wall., Kulu, N. W. Himalaya, India, collected by R. S. Troup, June 3, 1914. Specimen from the Pusa herbarium.

Through the courtesy of E. J. Butler one-half of the smaller specimen of *Peridermium indicum* has been deposited in the Pathological Collections of the Bureau of Plant Industry, United States Department of Agriculture.

POINTS OF SIMILARITY AND MEANS OF DIFFERENTIATION

Dietel (4) has already noted the similarity between the habit of *Peridermium kurilense* and the aecial stage of *Cronartium ribicola*. This resemblance is also to be observed between *P. indicum* and *C. ribicola*. The aeciospores of the two Asiatic specimens examined are very similar. Their relatively large size is obvious. Their position with respect to the specimen means of *C. ribicola* and *C. occidentale* may be determined easily by plotting their mean spore sizes on Figure 2 in the earlier paper (3). Both appear to be well without the *C. ribicola* range; but they might be confused with *C. occidentale*. The characters of the peridial cells are so different, however, that separation of the four species on the basis of these characters alone seems to be a relatively simple and certain method of diagnosis.

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NEW SPECIES OF CHALCID FLIES PARASITIC ON THE GIPSY-MOTH PARASITE, APANTELES MELANOSCELUS (RATZEBURG) [1]

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INTRODUCTION

In the course of investigations by the writer and S. M. Dohanian, of the gipsy moth and brown-tail moth project, the new species of chalcid flies described in this paper were found to be parasitic on the gipsy-moth parasite, *Apanteles melanoscelus* (Ratzeburg).

During the seasons of 1922, 1923, and 1924 an exhaustive study of the hyperparasites of *Apanteles melanoscelus* was made, not only to learn their biology and interrelationships, but also to determine the exact extent to which this useful parasite of the notorious gipsy moth, *Porthetria dispar* L., is subject to attack by secondary parasites. No less than 35 such enemies were discovered during the period of the investigations, only a few of which, however, were found to be of importance in reducing the activities of *melanoscelus*. *A. melanoscelus* is one of the most important of the several parasites of the gipsy moth which the Bureau of Entomology has successfully introduced and established in the gipsy-moth infested area of the New England States. It has two generations annually, both on the larvae of its host. Despite a long array of enemies, it has spread widely over the infested area and is aiding efficiently in reducing the ravages of the gipsy moth.

FAMILY PTEROMALIDAE

Coelopisthia scutellata, n. sp.

Female.—Length, 2 millimeters. Head transverse, much broader than thorax, and viewed from in front rather rounded, a little broader than long; eyes large, elongate-oval, bare, diverging a little below; entire head closely reticulately punctate, the face below antennae strongly receding and with very small but deeply impressed and closely set punctures; above the insertion of the antennae the punctures become gradually larger and more shallow toward vertex; clypeus more shining than lower part of face, its anterior margin weakly sinuate; scape of antenna slender, cylindrical, extending nearly to the median ocellus; pedicel long and slender, broadening apically, and distinctly longer than the two ring-joints and the first segment of funicle combined; first ring-joint very short, strongly tranverse; the second more than twice as long as the first, but still distinctly broader than long; funicle broadening very slightly apically; first funicle segment as long as broad, the following subequal and slightly broader than long; club acuminate, with the two basal segments about as long as the apical segments of funicle, the last segment shorter; lateral ocelli very slightly nearer to the median ocellus than to the eye margin; postocellar line at least one and one-half times the ocell-ocular line; pronotum closely punctate, a little more coarsely so than the head; mesoscutum very evenly punctate and sub-opaque, the punctures deeper and smaller than on the head, smaller anteriorly than behind; axillae and scutellum closely covered with minute, deep, almost

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confluent punctures, and opaque; the much finer punctation of the axillae and scutellum contrasts strongly with that of the mesoscutum; propodeum strongly punctate, the lateral folds complete, the median carina wanting; propodeal spiracles oval; neck of propodeum very short and provided with a conspicuous margined fovea at either side; pleura closely punctate and subopaque; anterior wings with postmarginal vein slightly shorter than stigmal vein, the latter distinctly less than half as long as the marginal vein; marginal cilia wanting; basal third of wing hairless, the remainder with very short hairs; abdomen ovate, pointed at tip, smooth, and polished. Head and thorax mostly bluish black; antennal scape yellow, remainder of antennae black; posterior margin of pronotum somewhat aeneous; scutellum, and the axillae to a smaller degree, bronzy green, contrasting strikingly with the color of mesoscutum; propodeum blue; anterior wings with a large deeply fuscous patch extending across wing from the base of marginal vein to a point beyond the end of stigmal vein; anterior coxae green or blue on the outer face; the middle pair mostly brownish, greenish only at base; the posterior pair blue; remainder of legs mostly brownish yellow; the posterior femora more or less dusky, especially along dorsal margin and at apex; the middle and hind tibiae brownish black, usually a little paler at base; and all tarsi pale yellow except for the dusky apical segment; abdomen dark aeneous, with slight purplish or greenish reflections at base.

Male.—In general like the female, but the face is bright green, the funicle of antenna is brown, and the fuscous spot in the anterior wing is much less pronounced.

Type.—Cat. No. 28123, U.S.N.M.

Type locality.—Franklin, N. H.

Host.—*Apanteles melanoscelus* (Ratzeburg).

Described from six females and six males reared under Gipsy Moth Laboratory No. 12599 C.

Hypopteromalus, inimicus n. sp.

Readily distinguished from *tabacum* (Fitch), with which it is frequently associated as a parasite of *Apanteles melanoscelus*, by the more slender antennal segments, the much darker coxae, the usually brighter green coloring of the thorax, and the longer and narrower abdomen of the female.

Female.—Length, 3 millimeters. Head strongly transverse, a little broader than the thorax, entirely deeply closely punctate; clypeus striate, its anterior margin deeply sinuate; eyes elongate-oval, moderate; antennae inserted a little above the level of the lower eye margin; scape slender, cylindrical, extending very nearly to the median ocellus; pedicel shorter than first segment of funicle; the two ring-joints very short, strongly transverse, the second only slightly longer than the first; funicle cylindrical; the first segment one and one-half times as long as broad; the following becoming gradually shorter, the sixth apparently very slightly broader than long; club a little wider than funicle, the segments subequal, broader than long; lateral ocelli nearer to the anterior ocellus than to the eye margin; postocellar line longer than ocell-ocular line but less than one and one-half times as long; pronotum strongly transverse, sharply margined anteriorly; mesoscutum, scutellum, and axillae closely punctate, the punctures on the scutellum and axillae being a little smaller than those on the middle of the mesoscutum; propodeum punctate medially, smooth outside the lateral folds, which are complete; median carina wanting; neck of propodeum large, conspicuous, transversely wrinkled; propodeal spiracles large, strongly elliptical; marginal vein slightly longer than postmarginal, the latter one and one-half times the length of stigmal vein; stigmal knob small; abdomen ovate, about as long as the thorax, pointed at apex, smooth and shining. Green; antennae brown, with scape and apex of pedicel yellow, the funicle and club yellowish beneath; tegulae yellow; wings hyaline; legs, except all coxae which are completely dark green, wholly yellow; posterior femora sometimes faintly brownish above toward base; abdomen dark green, dark cupreous medially.

Male.—Head and thorax more brilliant green than in the female; legs much paler yellow; antennae entirely yellow and more slender, with all the segments of funicle longer than broad, at least the first two, and sometimes the first four, segments twice as long as broad; abdomen with a median yellow spot covering apex of first segment and part of second.

Type.—Cat. No. 28122, U.S.N.M.

Type locality.—Boylston, Mass.

Host.—*Apanteles melanoscelus* (Ratzeburg).

Described from six females and five males, all reared from the above host; the type, allotype, and one paratype are from Boylston, Mass., and bear Gipsy Moth Laboratory No. 11239 a 2; four paratypes are from Pelham, N. H., three of these having been reared under Gipsy Moth Laboratory No. 11238 n and one under No. 11239 b; and the remaining three paratypes are from Melrose Highlands, Mass., two bearing Gipsy Moth Laboratory No. 10647 c and one Gipsy Moth Laboratory No. 10608.

FAMILY EULOPHIDAE

Dimmockia pallipes, n. sp.

Exceedingly similar to *secundus* Cwfd., from which it can be distinguished, however, by the conspicuous bronzy luster of the scutellum and head, and by the generally more delicate sculpturing of the scutellum.

Female.—Length, 1.7 millimeters. Head transverse, very slightly wider than thorax; the temples narrow; the cheeks rather broad; upper part of frons and the vertex mostly smooth and shining, with only a faint indication of sculpture; lower part of face weakly shallowly punctured, the punctures not especially distinct; malar space and cheeks very delicately reticulate, with a suggestion of vertical lineolation; eyes rather large, very broad, short-oval, bare; distance between the median and lateral ocelli subequal with ocell-ocular line; postocellar line at least one and one-half times the ocell-ocular line; antennae inserted about on a level with the lower eye margin; scape rather slender, subcylindrical, slightly thickened in the middle, and very nearly half as long as pedicel, funicle, and club united; pedicel a little longer than broad at apex, but distinctly shorter than first segment of funicle; the single ring-joint very short, strongly transverse; funicle 4-segmented, the first segment the longest, nearly one and one-half times as long as broad; the second, third, and fourth segments of about equal length, the third and fourth slightly broader than the second, usually subquadrate; club acuminate, 3-segmented, the segments poorly defined, the first and second transverse; pronotum conical, faintly punctured; mesoscutum shining, shallowly punctate, the punctures of the posterior part larger and deeper than on the anterior part; scutellum and axillae much more finely punctured, the punctures very small and elongate, and so arranged that the axillae and the scutellum laterally present a delicately lineolated appearance; medially on the scutellum the punctures are usually very shallow and rather indistinct; metathorax nearly smooth, shining; propodeum with the lateral and median carinae distinct, coarsely punctate between the lateral carinae, nearly smooth laterally; propodeal spiracle very small, circular; pleura shining, mostly delicately punctate; marginal vein nearly four times as long as stigmal vein, the postmarginal nearly twice as long as the stigmal; abdomen very slightly longer than thorax, ovate, pointed at tip, smooth and polished basally, faintly transversely lineolated on the posterior half. Green; head, scutellum, axillae, and postscutellum strongly bronzy; scape yellowish; rest of antennae dark brown; wings hyaline, veins pale brown; legs, including all coxae, entirely yellow; abdomen green, more or less aeneous posteriorly.

Male.—Like the female except in the following respects: Antennae with the scape shorter and broader; the pedicel stout, scarcely longer than broad at apex; the segments of the funicle elongate, twice or nearly twice as long as broad, each of the first three provided with a long slender branch, these branches subequal in length; club much broader than funicle and about one and one-half times as long as the last funicular segment; middle coxae slightly brownish at base; posterior coxae brownish black except at apex; abdomen with a pale yellow spot near base.

Type.—Cat. No. 28124, U.S.N.M.
Type locality.—Melrose Highlands, Mass.
Host.—*Apanteles melanoscelus* (Ratzeburg).

Described from nine females and seven males reared at the Gipsy Moth Laboratory; the type, allotype, and four paratypes bear Gipsy Moth Laboratory No. 11603 f 4 d; five paratypes were reared under No. 11603 a 3, and the remaining five under No. 11668 a 2.

EFFECT OF SOIL MICROORGANISMS ON PARAFFIN USED AS A COATING TO DECREASE THE INJURIOUS ACTION OF LEAD ARSENATE ON PLANT ROOTS [1]

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INTRODUCTION

Arsenate of lead mixed with soil was found by Leach (4) [2] to be a very effective compound for preventing the establishment of the Japanese beetle, *Popillia japonica* Newm., in the soil of certain limited areas such as cold frames. The arsenate acted as a stomach poison to the larvae. The arsenate in quantities toxic to the larvae was, however, injurious to certain plants. Particles of arsenate were coated with paraffin, various oils, fats, waxes, and other compounds in an effort to decrease the injurious action of the arsenate on these plants. Several coated lead arsenates were prepared and tested in the soil. Leach found that the arsenate coated with these materials was still as toxic to plants as the uncoated compound.

In view of the peculiar physical properties and the chemical inactivity of such materials as paraffin, it was not understood why lead arsenate coated with it was nevertheless injurious to plants. It was not unreasonable, however, to assume that some soil agent, either chemical or biological, had so modified the coating material as to liberate the arsenic. A study of the properties of paraffin led to the conclusion that the phenomenon was probably more biological than chemical. This conclusion was further strengthened when it was found that Söhngen (7) had described certain microorganisms which decomposed paraffin. An investigation was therefore undertaken to determine whether the soil microorganisms removed the paraffin coating from the arsenate particles.

METHOD OF STUDYING THE ACTION OF THE MICROORGANISMS

The actions of the soil microorganisms, the bacteria (including the actinomycetes), and the fungi on the paraffin-coated lead arsenate was studied in the laboratory under controlled conditions. Briefly stated, the method employed was as follows: 200 gm. of air-dried garden soil was placed in each of 140 glass jelly jars. Groups of 10 of these soil samples were mixed thoroughly with a definite quantity of either lead arsenate, lead arsenate coated with paraffin, lead arsenate coated with a mixture of paraffin and o-cresol, or paraffin finely ground with magnesium silicate.[3]

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Washington, D. C.

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Key No. K-185

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Ten samples of untreated soil served as a control. The materials containing arsenic were added to the soil at the rate of 0.4, 0.6, 0.8 and 1.0 gm. of lead arsenate per 200 gm. of air-dried soil; the paraffin, ground with magnesium silicate, was used at the rate of 0.6 gm., the quantity contained in the highest concentration of paraffin-coated lead arsenate. When all the soils were mixed, 20 c. c. of distilled water was added to each jar of soil to bring it to the optimum moisture content of 50 per cent saturation. Five larvae were embedded in each jar of soil. At intervals of 10 days 2 jars of each kind of treated soil and 2 of untreated soil were examined to determine the effect on the larvae,[4] the numbers of bacteria and fungi,[5] and the quantities of water-soluble arsenic produced.[6]

RESULTS OF EXPERIMENTS

The results of a single series of tests, summarized in Table 1, are typical of those obtained in the several other series. When uncoated lead arsenate was added to the soil in the proportions used in this test there was an incomplete mortality of the larvae at the end of 10 days. Coating the arsenate with an inactive material such as paraffin seemed to increase slightly the rate of larvicidal action of the arsenate.

The soil fungi were not stimulated to any considerable extent by the addition of lead arsenate; in the presence of paraffin the increase in fungi was almost proportionate to the amount of paraffin. Greaves (3) observed that certain salts of arsenic seem to exercise a stimulating action on bacterial activity. It was also observed in the experiment here reported that the bacteria were stimulated by the arsenate used alone. Paraffin did not stimulate the bacteria appreciably.

When lead arsenate was mixed with distilled water and the supernatant liquid analyzed for water-soluble arsenic, it was found to contain 1.35 mgm. of water-soluble arsenic pentoxide for each 0.4 gm. of the arsenate. The paraffin-coated arsenates gave only 0.39 mgm. and 0.50 mgm. of water-soluble arsenic pentoxide. When the uncoated lead arsenate was mixed with soil, more soluble arsenic was produced than when the arsenate was added to distilled water. This result is in agreement with that obtained by Stewart (8), who concluded that "the soil solution has a greater solvent power for lead arsenate than has pure water." It was also observed that the quantity of soluble arsenic produced by the paraffin-coated arsenate in the soil had increased over the quantity found in distilled water to an extent almost equal in some cases to that of the uncoated arsenate.

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[4] The larvae were removed and placed in fresh soil, and examined until their reaction could be determined.

[5] The number of bacteria (including the actinomycetes) was determined by the plate method described by Waksman and Fred (11). Ten grams of soil were shaken with 90 c c of sterile distilled water, and aliquot parts of this 1:10 dilution made up to dilutions of 1:10,000, 1:100,000, and 1:1,000,000. Five 1 c. c. samples of each of these higher dilutions were mixed thoroughly with 10 c. c. of melted sterile sodium-albuminate agar in sterile Petri dishes; after the agar had solidified the plates were incubated for 7 days at a temperature of 25° to 28° C. The colonies on each plate were counted and the number of bacteria (and actinomycetes) per gram of soil determined.

The number of fungi per gram of soil was determined by the plate method, the special medium recommended by Waksman (10) being used. The sterile melted special agar was inoculated in triplicate series with soil dilutions of 1:10, 1:100, and 1:1,000. The number of fungous "colonies" developing were counted after 48 hours.

[6] The quantity of water-soluble arsenic in coated and in uncoated lead arsenates was determined before they were mixed with the soil and at 10-day intervals after the mixing was done. To determine the arsenic before the mixing was done, 0.4 gm. of lead arsenate, coated or uncoated, was put into a 500 c. c. Erlenmeyer flask with 400 c. c. of distilled water, and allowed to stand for 24 hours at 25° C. The supernatant liquid was then filtered through a dry filter, and an aliquot part of the solution was analyzed according to the method recommended by the Association of Official Agricultural Chemists (1, p.59). The samples of soil were tested for soluble arsenic by mixing the 200-gm. sample with 1,000 c. c. of distilled water and analyzing the solution by the same method.

TABLE 1.—*Effect of coated and uncoated lead arsenate on the fungi, bacteria, and larvae in the soil and on the quantity of water-soluble arsenic produced*

Material added to soil	Quantity of material per 200 gms. of soil	Number of organisms found [a]				Quantity of water-soluble arsenic pentoxide produced—	
		Fungi per gram of soil	Bacteria per gram of soil	Larvae		In soil	In water
				Dead	Alive		
	Gm.	*Thou-sands*	*Millions*			*Mgm.*	*Mgm.*
Lead arsenate	0.40	7.6	51.6	5	5	2.32	1.35
	0.60	10.3	46.0	8	2	3.87	--------
	0.80	15.3	47.5	10	0	4.02	--------
	1.00	9.3	30.5	7	3	3.90	--------
Lead arsenate 100 grams and paraffin [b] 60 grams	0.64	35.6	4.7	8	2	2.20	0.39
	0.96	54.0	2.1	9	1	3.72	--------
	1.28	27.0	2.7	10	0	1.21	--------
	1.60	120.6	3.8	10	0	1.50	--------
Lead arsenate 100 grams and paraffin [c] 59 grams, and o-cresol [c] 1 gram	0.64	--------	--------	9	1	--------	0.50
	0.96	--------	--------	10	0	--------	--------
	1.28	--------	--------	10	0	--------	--------
	1.60	--------	--------	10	0	--------	--------
Paraffin	0.60	110.0	4.5	0	10	0.42	0.00
Untreated (control)	0.00	11.3	2.0	3	12	0.42	0.00

[a] The bacteria, fungi, and water-soluble arsenic were determined 40 days after the beginning of the experiment, the effect on the larvae was determined after 10 days.
[b] As a coating.
[c] Paraffin and o-cresol mixture used as a coating.

DISCUSSION OF RESULTS OBTAINED BY OTHER INVESTIGATORS

The results of these experiments, when studied in conjunction with those obtained by other investigators, indicate that soil microorganisms, especially the soil fungi, remove the paraffin from the lead arsenate particles in the soil. A number of years ago Rahn (5) called attention to the presence in the soil of a species of Penicillium capable, when grown in cultural solutions containing paraffin as the only organic constituent, of utilizing paraffin in its metabolism. Gainey (2, p. 362) observed that an enormous development of certain species of fungi occurred parallel with the progressive disappearance of active nitrogen and of paraffin in the soil. He concluded that it was not unreasonable to assume that the enormous growth of fungi, observed where paraffin was added, utilized the paraffin as a source of carbon and of energy; also that active nitrogen which disappeared was utilized as a source of nitrogen in the metabolism of the fungi. In addition to the Penicillia, the Aspergilli, Cladosporia, Trichodermae, and the Mucorales have been isolated from the soil by Waksman (9) and others.

Numerous experimenters have reported that various species of Aspergillus and Penicillium produce organic acids, such as oxalic, citric, and other organic acids in the course of their metabolism. The removal of the chemically inert paraffin coating and the formation of these acids on the surface of the lead arsenate particles would tend to increase the quantity of water-soluble arsenic produced by the coated material.

Russell (6, p. 31) remarks that "a large number of soil bacteria have now been isolated that can decompose phenol, meta, para, and ortho cresol, and are able to use these substances as the sole sources of energy for their life processes." Söhngen (7) found that certain

soil bacteria also decompose and use similarly benzene, petroleum, paraffin oil, and paraffin. Soil bacteria which are able to decompose and derive their energy from naphthalene and toluene have also been isolated. The results obtained by the writer, however, seem to indicate that the bacteria are not so important as the fungi in decomposing the paraffin coating of the arsenate in the soil.

The soil microorganisms apparently have the ability to decompose the various oils, fats, waxes, and paraffin which were used by Leach (*4*) to decrease the injurious action of lead arsenate on certain plant roots. It seems futile, therefore, to attempt to protect the plants from arsenic injury by coating the poison with these materials.

SUMMARY

Coating lead arsenate with paraffin and with various oils and fats did not decrease the injurious action of the insecticide in the soil or reduce its toxic effect on plants. A study was made of the action of soil bacteria and soil fungi on lead arsenate coated with paraffin, to determine whether these organisms were responsible for this injurious action. It was found that soil fungi were stimulated by paraffin. Various species of fungi are known which are capable of utilizing paraffin in the course of their metabolism and converting it into some organic acid. The soil bacteria were less active than fungi in decomposing the paraffin coating. The conversion of the paraffin coating into organic acids may explain the injurious action of the coated insecticide in the soil.

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THE DETERMINATION OF ORGANIC MATTER IN SOILS BY MEANS OF HYDROGEN PEROXIDE [1]

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INTRODUCTION

The organic matter of soils is generally determined by converting it to carbon dioxide by means of moist and dry combustion. The carbon dioxide thus evolved is then calculated to organic matter by the use of a conventional factor, 0.471. It has been found, however, that the percentage of carbon in the organic matter of soils varies widely. Some doubt, therefore, is cast on the accuracy of the combustion methods, as applied to any particular soil. For this reason a new method which does not involve the use of an empirical factor is desirable.

Inasmuch as several investigators have reported that a large part of the soil organic matter is decomposed by hydrogen peroxide, it seemed possible that the organic matter might be determined by treating a sample of soil with hydrogen peroxide and noting the loss in weight. A treatment with hydrogen peroxide, unlike treatment by the combustion methods, would not be expected to affect the combined water content or appreciably change the weight of the inorganic material.

In testing the use of hydrogen peroxide for determining organic matter, information has been obtained regarding the decomposability of various kinds of soil organic matter; and also, concerning the accuracy of the conventional factor, 0.471, when applied to the organic matter of different soils.

REVIEW OF PREVIOUS WORK

Coppenrath, Hasenbäumer, and König (2)[2] and also May and Gile (6) have shown that the quantity of oxygen set free from the hydrogen peroxide used is roughly proportional to the quantity of organic matter in the soil.

König, Hasenbäumer, and Grossmann (5) found that hydrogen peroxide converted from 60 to 90 per cent of the total carbon of the soil to carbon dioxide.

Peterson (7), when studying the organic phosphorus of soils, was the first to determine the loss in weight of the soil material caused by the destruction of the organic matter by hydrogen peroxide. His method consisted in digesting the soil with dilute hydrogen peroxide in a covered beaker until the weight became constant. On comparing the loss in weight with the quantity of organic matter determined by combustion, he concluded that hydrogen peroxide

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Journal of Agricultural Research,
Washington, D. C.

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destroyed about 90 per cent of the soil organic matter. Peterson did not determine whether any carbon was left in the digested residues; thus it is not known whether failure of the two methods to agree was due to incomplete oxidation or to organic matter which has a different percentage of carbon than that assumed when the conventional factor is used.

Robinson (9) proposed the use of hydrogen peroxide in the mechanical analysis of soils to destroy the organic matter which prevented the deflocculation of the soil particles. He observed that the destruction of the organic matter was not complete. Later he and Jones (10) found that cotton fiber, crude fiber from straw, etc., were not decomposed by hydrogen peroxide under their experimental conditions, whereas humus, or organic matter which had been extracted from soils by ammonia was completely decomposed. They therefore proposed the use of hydrogen peroxide to differentiate between the humified and nonhumified organic matter of the soil. The humified organic matter was determined by subtracting the ignition loss of the soil after treatment with hydrogen peroxide from the ignition loss of the untreated soil. They found that the humified organic matter varied from 3.4 to 57.5 per cent in the mineral and peat soils of Wales, and that it constituted from 42.3 to 82.3 per cent of the total organic matter, as determined by the loss on ignition. The percentage of organic matter destroyed is doubtless somewhat too low, since the loss on ignition is almost invariably greater than the organic matter contained in a soil. Robinson and Jones took precautions to minimize this error by selecting soils free from carbonates and, presumably, low in combined water, since they were of low clay content.

THE HYDROGEN PEROXIDE METHOD

In the hydrogen peroxide method the soil organic matter is determined from the loss in weight caused by digesting the soil with hydrogen peroxide. The method adopted in this investigation is described as follows:

About 1 gm. of ordinary soil or 0.2 gm. of peat was accurately weighed into a tall 250 c. c. beaker, to which 10 c. c. of water and 10 c. c. of 30 per cent hydrogen peroxide [3] were added. The beaker was covered and watched for a few minutes. If the reaction was not too vigorous,[4] the beaker was placed on a steam bath and the contents digested until no more bubbles evolved. If the reaction is too vigorous the method can not be applied without modification to the particular soil. The digestion was usually complete within 1 hour, the peats and mucks requiring more time. After the bubbles ceased no more hydrogen peroxide remained, as is shown by the permanganate test. The beaker was frequently manipulated during the digestion in order to bring the lighter particles of organic matter in contact with the solution as much as possible. After it had settled the solution was filtered through a thick and compact asbestos pad in a special Gooch crucible. Filtration is necessary to separate

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[3] This hydrogen peroxide contained from 30 to 33 per cent of H_2O_2. The ash content of 0.001 per cent was disregarded. A small quantity of preservative present contained nitrogen and carbon. Suitable allowances were made for the nitrogen and carbon in some of the details of this study.

[4] A violent reaction with the evolution of heat shows the presence of manganese dioxide (MnO_2) or chromium sesquioxide (Cr_2O_3) in the soil. If the quantity of manganese dioxide is small (say less than 0.25 per cent), it can be destroyed by evaporating with a very small excess of oxalic acid, and the soil may then be treated as indicated above. If much manganese dioxide is present, this method is not applicable.

from the residue some soluble, undecomposed organic matter and ammonium salts which are always present in solution. The residue was washed a little, and the filtrate and washings were evaporated in a platinum dish, ignited and weighed. This was necessary since the weight of the ash of the soluble matter must be added to the weight of the residue.

The filtrate should be practically clear. Occasionally heavy clay soils will give a turbid or muddy filtrate. If passing the suspension through the pad a second time without allowing the pad to become dry fails to remove the suspended matter, certain corrections must be made. The weight of the ignited suspended matter can not be directly added to the weight of the residue in the Gooch crucible, since the clay loses about 10 per cent of combined water on ignition. Separate estimates of clay and soluble inorganic matter must be made, so that the ignited clay can be calculated to its weight at 110° C. This is done as follows: The filtrate is made up to a definite volume and is allowed to settle. The clear, upper half is evaporated, ignited, and weighed, giving one-half of the weight of the soluble inorganic matter. The turbid, lower half is treated in the same manner. The difference between these two weights gives the weight of ignited clay. This weight is increased by one-ninth to give the weight of the clay dried at 110° C. The corrected weight of the clay and the determined weight of the soluble, inorganic material are then added to that of the filtered residue.

The insoluble matter remaining on the pad was dried for 18 hours at 110° C. and weighed. The moisture determination in the untreated soil material was made at the same time. The difference in weight between the moisture-free material and the total weight of the dried residue plus the ash obtained from the solution was calculated as organic matter.

The concentrations and quantities of peroxide most favorable for the complete decomposition of the organic matter were selected after several trials. The results of these trials on four soils, high and low in organic matter, are given in Table 1.

TABLE 1.—*Effect of hydrogen peroxide in different concentrations on the decomposition of organic matter in different types of soil*

Types of soil	Depth at which soil sample was taken	Decomposition solution used		Organic matter decomposed
		30 per cent H_2O_2	Water	
	Inches	*C. c.*	*C. c.*	*Per cent*
Velasquez loam	12 to 32	10		12.64
Do	do	10	10	12.94
Do	do	10	50	12.73
Do	do	20	20	13.00
Do	do	40	40	13.12
Ontario loam	0 to 12	10		3.01
Do	do	10	10	2.82
Do	do	10	50	3.19
Carrington loam	do	10	10	3.93
Do	do	20	20	3.63
Do	do	40	40	3.58
Peat	46 to 70	10		87.1
Do	do	10	50	90.7
Do	do	20	20	86.6
Do	do	40	40	89.9

It is evident that accurate determinations of the dried weights of the soil and digested residues are very important. Considerable difficulty was experienced in drying the residue to constant weight in an ordinary Gooch crucible on account of the very hydroscopic nature of some of the materials. By providing the crucibles with tightly fitting ground-on covers, satisfactory results were obtained. Pyrex-glass crucibles were used.

The quantities of organic matter destroyed by hydrogen peroxide show no consistent variation with increasing quantities or concentrations of the reagent. The differences are about the same as are obtained between duplicates, although the duplicates agreed more closely in subsequent work, as the technique improved with practice. Evidently the quantity of hydrogen peroxide specified in the procedure was sufficient to produce maximum decomposition of the organic matter.

Although tests showed that nothing was to be gained by the use of different concentrations of hydrogen peroxide, it was not known whether more organic matter would be decomposed by additional treatments with the reagent. Two different soils were therefore subjected to successive treatments with 20 c. c. of the 15 per cent hydrogen peroxide used in the procedure. The results are shown in Table 2.

TABLE 2.—*Effect of successive treatments by hydrogen peroxide on quantity of organic matter decomposed in soils of different types*

Type of soil	Number of treatments with H_2O_2	Organic matter de-composed
		Per cent
Velasquez loam	1	12. 64
Do.	2	12. 81
Do.	3	12 65
Peat	1	87. 1
Do.	2	87. 8
Do.	3	90. 7

The data in Table 2 show no consistent increase in the quantities of organic matter decomposed by additional treatments with hydrogen peroxide. Later work showed that, under an excessively prolonged treatment, slightly more organic, or carbonaceous matter could be decomposed by hydrogen peroxide, but the process was too tedious to be adopted as a practical method. It is thought that the procedure adopted gives as complete decomposition of the organic matter as is practicable.

COMPOSITION OF SOIL MATERIAL MADE SOLUBLE BY HYDROGEN PEROXIDE

In describing the hydrogen peroxide method it was stated that the filtrate from the peroxide digestion contained soluble matter for which corrections were made.

About half of the matter made soluble was volatile at a dull-red heat. Part of the volatile matter was obviously organic, but its nature was not determined. König, Hasenbäumer, and Grossmann (5) found that formic and acetic acids were formed under similar

conditions.   Ammonium salts also formed part of the volatile matter; in fact analyses of several of the salt solutions showed that practically all the soil nitrogen was converted into ammonium salts.

It is clear that the soluble, volatile matter should constitute a part of the organic matter indicated by the loss in weight of the material when treated with hydrogen peroxide.   Thus, the weight of the residue from the peroxide digestion is not corrected for the soluble volatile matter.   The ash remaining after the volatile matter is driven off, however, should be added to the weight of the residue. This correction for the ash, always made, ranged from 0.11 to 2.04 per cent, and was important in most cases.   The ash does not seem to result from the solution of the soil inorganic matter, since the quantity of ash is not proportional to the quantity of hydrogen peroxide used, to the volume of the solution, or to the time of digestion.   Evidently the ash comes from the organic matter which is decomposed, since different soils usually give quantities of ash proportional to the quantities of organic matter present.   Furthermore, the ash yielded by different quantities of a particular soil is proportional to the quantity used.

The partial composition of the soluble inorganic matter present in the solution resulting from the digestion of three different soil materials with hydrogen peroxide is given in Table 3.

TABLE 3.—*Partial composition of inorganic matter in soils of different types made soluble by hydrogen peroxide (expressed as a percentage of the weight of soil)*

Type of soil	SiO$_2$	Al$_2$O$_3$+ Fe$_2$O$_3$	CaO	MgO	MnO
	Per cent	Per cent	Per cent	Per cent	Per cent
Carrington loam	0.112	0.048	0.064	0 021	0 002
Hagerstown loam	.038	.022	.388	.044	.040
Velasquez loam	.073	.015	.168	.013	N. D.

Although the soluble inorganic matter of soils contains sulphates and chlorides also, the quantities of these elements were not determined.   Evidently the constituents of the ash vary considerably in different soils.   Lime is obviously one of the chief constituents. König, Hasenbäumer, and Grossmann (5) also found lime to be the chief soluble constituent under similar conditions.   In some soils manganese may be the most important constituent of the ash. This was found to be so in the case of the Blakely and Amito loams.

It seems likely that most of the soluble inorganic matter has its origin in the destruction of the organic matter of the soil.   In a sense it might be considered a part of the organic matter.   However, it is left in the soil when the organic matter decays, and in all combustion methods it is neglected.   It seemed best, therefore, to deduct the weight of the soluble, inorganic matter from the entire loss in weight caused by the digestion of the organic matter by hydrogen peroxide.

EXTENT TO WHICH CERTAIN KINDS OF ORGANIC MATTER ARE DECOMPOSED BY HYDROGEN PEROXIDE

Before determining the decomposability of the total soil organic matter it seemed best to study the action of hydrogen peroxide on some simpler organic materials, especially in view of the fact that

Robinson and Jones (10) reported that unhumified organic matter, such as cotton fiber and crude fiber, were not attacked by peroxide.

Preliminary tests made in the laboratory showed that the conclusion of Robinson and Jones regarding the nondecomposability of unhumified organic matter is true only under certain conditions. It was found that 15 per cent hydrogen peroxide had practically no effect on cotton fiber and crude fiber, provided they were digested in the absence of other material. However, when the digestion was carried out in the presence of soil both the cotton fiber and crude fiber were destroyed almost quantitatively.

Inasmuch as other investigators have found that all the soil organic matter is not decomposed by hydrogen peroxide, such material as charcoal, coal, and graphite were included in the substances tested, since they are likely to occur in soils and presumably are difficult to oxidize. Since the presence of soil material has such a marked effect on the decomposability of cotton fiber and crude fiber, the tests of the decomposability of various kinds of carbonaceous substances were made on samples mixed with a portion of the Orangeburg fine sandy loam subsoil. The proportion of organic matter decomposed was determined from the loss in weight of the sample in some cases, and in other cases, by measuring the carbon content before and after treatment. The results are given in Table 4.

TABLE 4.—*Decomposition of various kinds of carbonaceous matter by 15 per cent hydrogen peroxide in the presence of soil*

Kind of carbonaceous matter	Quantity decomposed
	Per cent
Coal, anthracite (100-mesh screen)	10 2
Coal, bituminous (100-mesh screen)	7. 8
Coal, cannel (100-mesh screen)	14. 2
Charcoal in contact with soil but a short time [a] (100-mesh screen)	29. 8
Charcoallike particles separated from soil [b] (100-mesh screen)	89. 4
Cotton fiber	99. 6
Crude fiber from wheat straw	98. 4
Graphite, natural (100-mesh screen)	0. 0
Graphite schist separated from soil	0. 0
Humus, extracted by ammonia	100. 0
Lignite (100-mesh screen)	98. 7
Lubricating oil	66. 4
Peat, mosslike	99. 5
Peat, dark brown	97. 0

[a] Charcoal particles were separated from a cultivated soil where a large brush pile of hardwood had been burned about nine months before the charcoal was gathered.

[b] Composite of several very small samples of charcoallike particles, separated from a number of soils and subsoils by Schreiner and Brown (11)

Table 4 shows that coals and graphite, in particular, were hardly attacked, and that charcoals were decomposed in varying degrees. More than half of a lubricating oil was destroyed, and the other substances were decomposed almost quantitatively.

It is certain that in the method adopted hydrogen peroxide can not be used to distinguish humified and nonhumified organic matter. Brown peat, mosslike peat, cotton fiber and crude fiber were almost completely decomposed under the conditions of this study. It is true that Robinson and Jones used weaker hydrogen peroxide and allowed a shorter time for digestion; but it is believed that the discrepancy between their results and those given in Table 4 is due

chiefly to the fact that they used hydrogen peroxide alone, whereas in the experiments described in this paper soil material, acting as a catalyzer, was always present during the digestion.

From these data it would be expected that the procedure adopted would result in the decomposition of all except the most resistant carbonaceous matter found in soils. The results for charcoal tend to show that different charcoals vary considerably in decomposability.

RESULTS OF ANALYSES OF DIFFERENT SOILS

A satisfactory procedure having been established, samples of material from a number of different soil types were analyzed for organic matter by the peroxide method.

Samples were selected from several soil types which are very high and several which are very low in organic matter. Three soil colloids and several very clayey subsoils were also selected with a view to studying the possible effects of colloid surfaces and of a high percentage of combined water on the accuracy of the method. To test the possible effect of the action of hydrogen peroxide on soil mineral matter, materials from several very deep subsoils low in organic matter were included. It was thought that if any oxidation of the mineral matter took place it would be greatest in the deep subsoil materials where natural oxidation is not so pronounced as in surface layers. Duplicate determinations were made on all samples. After the material had been treated with hydrogen peroxide, the residues were analyzed for undecomposed organic matter by the combustion method,[5] and also for carbonates.[6] These results are given in Table 5.

The results for organic matter, in Table 5, have not been corrected for the carbonates found in the residues. In each case the combustible matter found in the residue has been calculated to carbon, since it seemed likely that it contained a high percentage of carbon. All quantities are expressed as percentages of the weight of the original material used, dried at 110° C.

It is apparent, from columns 3 and 4 in Table 5, that the duplicate results obtained by the peroxide method agree about as well as those of other constituents, obtained by ordinary methods of soil analysis. To get closely agreeing duplicates it is necessary to observe extreme care in getting the dried weights. The sample should be weighed from a weighing bottle, and the Gooch crucible should be covered promptly just before transferring it from the drying oven to the desiccator.

The carbon dioxide of the carbonates in the residue evidently results from the action of hydrogen peroxide on the organic matter. The carbon dioxide, shown in the last column of Table 5, should properly be added to the organic matter found, since the residue is too heavy by just that quantity. However, it is apparent that such a correction would not be of any importance ordinarily. In 15 of the 26 materials analyzed this correction would be less than 0.15 per

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[5] An electric combustion furnace giving a maximum temperature of 1,100° C. was used. With a quartz combustion tube it was possible to heat the sample nearly to the maximum temperature, thus insuring complete combustion of graphite and other difficultly combustible carbon of low combustibility. The mixed gases from the furnace were dried by phosphorus pentoxide both before entering and in the carbon dioxide absorption apparatus.

[6] Only one soil contained carbonates before treatment. A small quantity of carbonates is formed by the action of the hydrogen peroxide on the soil.

cent of the dry weight of the sample analyzed. The values given for organic matter in columns 3 and 4 of Table 5 have not been corrected for carbonates.

TABLE 5.—*Organic matter in soils of various types as determined by the hydrogen peroxide method and the undecomposed carbon and the carbonates in the residues*

Type of soil material	Depth at which soil sample was taken	Quantity of organic matter as determined by decomposition with H_2O_2 (duplicate results)		Quantity of combustible matter calculated as carbon, in residue from H_2O_2 treatment	Carbonates in the residues from the H_2O_2 treatment (calculated as CO_2)
	Inches	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Amito loam, Mississippi	0–12	2.35	2.39	0.48	0.06
Do	12–36	1.63	1.73	37	.02
Blakeley loam, Georgia	0–12	2 14	2.24	.23	.24
Carrington loam, Iowa	0–12	[1] 3.59	[1] 3.59	30	.00
Carrington loam colloid, Iowa	0–12	10.17	10.08	41	.06
Cukre clay, Nicaragua, Central America	0–5	8.89	8.89	.67	.16
Do	5–60	2 12	2.04	.28	.17
Hagerstown loam, Pennsylvania	0–8	4.78	4.67	1.90	.19
Houston black clay, Texas	0–12	3 39	3.49	.60	.20
Mosslike peat, Florida	0–8	95.60	95.50	.25	.02
Muck, Indiana	0–6	22.45	22.36	1.29	.75
Do	6–12	12 17	12.38	60	.33
Ontario loam, New York	0–12	[1] 3 00	[1] 3.00	.06	.05
Orangeburg fine sandy loam, Mississippi	10–36	30	.41	.06	.00
Orangeburg fine sandy loam colloid, Mississippi	0–10	3.62	3.58	.38	.03
Lenox stony loam, Massachusetts	0–6	4.83	4.83	.50	.04
Peat, Florida	46–70	90.70	89.90	1.45	.37
Palmar clay, Colombia, South America	0–14	6.67	6.76	.26	.15
Do	14–60	1 06	.95	.07	.06
Do	60–72	.84	.74	.10	.06
Do	72–96	.60	.61	.06	.02
Do	96–120	.51	.51	04	.00
Ranger loam, Georgia	0–10	2.00	1.80	.33	.00
Sharkey clay, Mississippi	0–4	3 98	3.72	.44	.21
Velasquez loam, Guatemala, Central America	12–32	2 12.90	2 12 90	.73	.27
Wabash silt loam colloid, Nebraska	0–15	4.60	4.59	.52	.09

[1] Average of 6 determinations.   [2] Average of 12 determinations.

The carbonaceous matter not oxidized bears no constant relation to the total quantity of organic matter present. In a few cases it is inconsiderable when calculated as carbon, amounting to less than 5 per cent of the total organic matter. In most of the materials, however, the undecomposed carbonaceous matter is considerable. It is evident, therefore, that the hydrogen peroxide method does not give an accurate determination of the total organic matter, that is, of the total quantity of carbon, or carbon compounds exclusive of carbonates.

NATURE OF THE UNDECOMPOSED ORGANIC MATTER

Since considerable quantities of organic matter or some substances containing carbon are not decomposed by the hydrogen peroxide treatment, the identification of these carbon-containing substances in the residues is important. If the residue after digestion contains only a definite kind of organic matter, the hydrogen peroxide method could be used to differentiate the two kinds of organic matter present in soils, and also to determine the quantity of the decomposable portion.

The quantity of organic matter left undecomposed is not dependent primarily on the state of subdivision, since the organic residue from the mosslike peat is less than the organic residues from the three soil colloids.

General knowledge concerning the soils treated was of little value in determining the nature of the undecomposed organic matter, except in the case of the sample of Hagerstown loam. This sample, taken from the "charcoal plot" of the Pennsylvania Experiment Station, was known to contain charcoal; and it will be noted in Table 5 that the quantity of undecomposed carbon is exceptionally large in this soil (*13*). The Ranger loam and the Lenox stony loam are peculiar soils in that they are derived from graphite schist. In samples of these soils the quantities of undecomposed carbonaceous matter are not large; hence it is improbable that graphite constitutes any considerable part of the carbon in the undecomposed residues of the other soil materials.

The preliminary work on the decomposition of various organic materials indicated that graphite, coal, and certain kinds of charcoal, if present in soils, would be found in the part not attacked by hydrogen peroxide. However, it is not to be expected that graphite, widely distributed in small quantities, would occur in anything more than traces in most soils. Coal particles would be restricted to coal-bearing regions. Because of the prevalence of forest fires, charcoal might be present in almost all soils. The decomposability of charcoals, however, is evidently variable.

Schreiner and Brown (*11*) found coallike and charcoallike particles in all of 34 soils examined by them, and also in samples from a number of deep subsoils. They point out that numerous forest fires furnish an ample source of charcoal, but add that it is not known how long the charcoal persists in the soil. White and Holben (*14*) showed that charcoal had persisted in the charcoal plot of the Pennsylvania experimental plots for more than 68 years. This particular plot was once the site of a charcoal furnace, and the soil on it now contains about 4 per cent of carbon. White and Holben found that charcoal in small quantities was a general constitutent of all the Pennsylvania plots, and they consider charcoal a general, though small, constituent of soils in the eastern United States.

The residues from the hydrogen peroxide treatment were examined microscopically for evidence as to the nature of the undecomposed organic matter. Occasionally particles resembling roots were observed, but these particles seemed to be mere outlines and entirely different in appearance from roots not treated with hydrogen peroxide. In the Hagerstown loam, black particles, evidently charcoal, could be seen. The black particles gather on the sides of the beaker during the digestion. Charcoal can be distinguished by a test, devised by J. G. Smith of this laboratory, which consists of drawing a spatula firmly over a small quantity of soil material which has been placed on a clean, white sheet of paper. Charcoal gives a characteristic black streak. This may be confirmed by burning the black particles and by microscopic examination. White and Holben (*14*) recommend testing the soil for charcoal by stirring the soil in water; the charcoal particles will then rise to the surface.

Apparently there are forms of carbonaceous matter in the digested residues which do not possess the characteristics of charcoal, coal, or graphite. The residues from the peat and muck soil contained small quantities of a waxy substance which yielded dense, white fumes on burning. The odor and the fumes given off resembled those of paraffin. None of the other residues gave off white fumes on

burning. The fact that they blackened momentarily, indicated that a small quantity of organic matter was distributed through the residues. This momentary blackening may have been due to a trace of soluble organic matter which was not thoroughly removed by washing, since König, Hasenbäumer, and Grossmann (5) have found that acetic and formic acids are formed by the action of hydrogen peroxide on soil organic matter.

It is desirable to know the percentage of carbon in the carbonaceous matter of the undecomposed residues. It was found that further oxidation of the carbonaceous matter could be obtained by prolonged boiling of the residues in a closed flask with hydrogen peroxide. The flask was connected with a Knorr carbon dioxide apparatus and the carbon dioxide given off was weighed. Ten cubic centimeters of the 30 per cent peroxide was added and the contents of the flask were gently heated and finally boiled until no more carbon dioxide was given off, the process requiring several hours. Ten cubic centimeters more of peroxide was added, and this procedure was repeated until no more carbon dioxide could be obtained from the residues, 6 to 10 treatments being required. The boiled residue was then collected on a Gooch crucible, dried, and weighed, and the loss in weight noted. From the loss in weight of the sample and the weight of the carbon dioxide evolved the percentage of carbon in the carbonaceous matter destroyed was calculated. The carbon remaining after the prolonged boiling with hydrogen peroxide was determined by subtracting the quantity of carbon thus oxidized from the quantity of carbon present in the residue obtained in the standard method. These determinations, together with the carbon content of the residues from the first hydrogen peroxide treatment, are given in Table 6. Unless otherwise noted, the percentages are calculated on the weight of the oven-dried material rather than on the weight of the residue.

TABLE 6.—*Effect of prolonged boiling with hydrogen peroxide on the carbonaceous matter that remains after soils are treated by the standard peroxide method*

Type of soil or colloid	Quantity of carbon left after treatment by the standard peroxide method	Quantity of carbonaceous matter destroyed by prolonged boiling with hydrogen peroxide	Percentage of carbon in the carbonaceous material destroyed by prolonged boiling with hydrogen peroxide	Quantity of carbon left after prolonged boiling with hydrogen peroxide
	Per cent	*Per cent*		*Per cent*
Hagerstown loam	1.90	2.01	90	0.09
Carrington loam colloid	.41	.22	95	.20
Muck (surface layer)	1.29	.52	73	.91

The quantities of carbon in the residues before and after prolonged boiling show that practically all the carbonaceous matter in the Hagerstown loam was decomposed by this treatment and that only from one-half to one-third of that contained in the Carrington loam colloid and muck was digested. The matter decomposed, being highly carbonaceous, is probably charcoal, while that unattacked is, presumably, not charcoal. These assumptions are supported by the fact that the carbon in the sample of Hagerstown loam, a soil material containing a large quantity of charcoal, was practically all

oxidized.   Furthermore, a test showed that finely ground, nearly
.fresh charcoal was completely decomposed by prolonged boiling with
peroxide.

It would seem from the above determinations that charcoal, or
some material very high in carbon, usually constitutes a part of the
carbonaceous matter that is left in the first treatment with hydrogen
peroxide.   Other more resistant carbonaceous substances may also
be present.   It is not known whether this resistant matter is high or
low in carbon.   The residues obtained in the analyses of Hagerstown
loam, Houston black clay, and Sharkey clay by the standard method
were found to contain insufficient nitrogen to give a test.   It is
probable, therefore, that the resistant matter contains no nitrogen.

It is not possible to state definitely the nature of the carbonaceous
matter which resists decomposition by hydrogen peroxide in the
adopted procedure.   It is quite certain, however, that all organic
matter of the nature of humus, crude fiber, and other cellulose ma-
terial is decomposed.   This is shown in the preliminary tests of
these materials, and also by the fact that the residues from the peat
and mosslike peat were very small.   Preliminary tests with the
adopted method indicate also a partial decomposition of charcoal
or coallike material.   From the microscopic examination it seems
that a considerable part of the undecomposed organic matter in most
of the materials examined may be charcoal, and graphite in two of
the samples.   In two cases a paraffinlike substance in the residues
was indicated by ignition.   Prolonged boiling with hydrogen per-
oxide also indicated that charcoal or a substance very high in carbon
contained practically all the carbon of the undecomposed residue in
one sample, and from one-half to one-third of the carbon in the
residues of two other soils.   Apparently the organic residues obtained
from different soils contain different forms of carbon, and more
than one kind of carbonaceous matter may be present in a single
residue.   In general, however, charcoal seems to be the most com-
mon carbonaceous constituent.

COMPARISON OF THE HYDROGEN PEROXIDE AND COMBUSTION METHODS OF DETERMINING ORGANIC MATTER

A comparison of the quantity of organic matter destroyed by the
hydrogen peroxide method with the total organic matter determined
by combustion is important.   Although the former method is not
accurate in determining the total organic matter in soils, since con-
siderable carbon is found in the residues, the latter method is likewise
inaccurate because of the uncertainty of the use of the factor for a
particular soil.   The hydrogen peroxide method has an advantage in
that it gives a direct determination of the weight of the organic
matter destroyed; and, since the quantity of undecomposed car-
bonaceous matter is small in many cases, it would seem that this
method might give results as near the correct value as would the
combustion methods.

Combustion determinations of organic matter were made on all the
soils and colloids used in this investigation.   The results of these
combustion determinations are given in Table 7.   In this table the
organic matter determined by the hydrogen peroxide method is cor-
rected for the carbonates present in the residues.   The quantity of
carbonaceous matter not decomposed by the peroxide is calculated

as carbon. The sum of the organic matter determined by hydrogen peroxide plus the quantity of carbon found in the residues is given for comparison with the combustion and hydrogen peroxide results. All percentages are calculated on the dry weight of the untreated material.

TABLE 7.—*Comparison of organic matter of different soils as determined by the combustion method and the hydrogen peroxide method*

Type of soil and colloid	Depth at which soil sample was taken	Quantity of organic matter determined by combustion ($CO_2 \times$ 0 471)	Quantity of organic matter determined by hydrogen peroxide	Carbonaceous matter obtained from residue of peroxide digestion calculated as carbon	Organic matter obtained by hydrogen peroxide method plus the carbon in the residue	Organic matter as determined by combustion method compared with organic matter determined by hydrogen peroxide method plus the carbon in the residue
	Inches	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Amito loam	0–12	3.34	2.43	0.48	2.91	0.43
Do	12–36	2.30	1.70	.37	2.07	.23
Blakeley loam	0–12	2.91	2.43	.23	2.66	.25
Carrington loam	0–12	4.00	3 59	.30	3.89	.11
Carrington loam colloid	0–12	11.57	10.19	.41	10.60	.97
Cukre clay	0–5	9.56	9 05	.67	9.72	−.16
Do	5–60	2.49	2.25	.28	2.53	−.04
Hagerstown loam	0–8	9.22	4.92	1.90	6.82	2.40
Houston black clay	0–12	4.20	3.64	.60	4.24	−.04
Lenox stony loam	0–7	5.22	4.87	.50	5.37	−.15
Mosslike peat	0–8	85.60	95.57	.28	95.85	−10.25
Muck	0–6	24.47	23 16	1.29	24.45	.02
Do	6–12	14.09	12.61	.60	13.21	.88
Ontario loam	0–12	3.07	3.05	.06	3.11	−.04
Orangeburg fine sandy loam	10–36	.45	.36	.06	.42	.03
Orangeburg fine sandy loam colloid	0–10	4.28	3 63	.38	4.01	.27
Peat, from Florida	46–70	97.49	90.67	1.45	92.12	5.37
Palmar clay	0–14	7.38	6 87	.27	7.14	.24
Do	14–60	.87	1.06	.07	1.13	−.26
Do	60–72	.73	.85	.10	.95	−.22
Do	72–96	.68	.63	.06	69	−.01
Do	96–120	.44	.51	.04	.55	−.11
Ranger loam	0–10	2.24	1.90	.32	2 22	.02
Sharkey clay	0–4	4.19	4.01	.44	4.45	−.26
Velasquez loam	12–32	15.06	13.17	.73	13.90	1.16
Wabash silt loam colloid	0–15	5.54	4 69	.50	5.19	.35

A comparison of columns 3 and 4 of Table 7 shows that the combustion method gives higher results than the hydrogen peroxide method in 22 of the 26 determinations, the differences being considerable in most cases. In 4 cases the hydrogen peroxide results exceed those obtained by the combustion method.

In some cases the ignition loss indicates which result is nearer the true value. In any case the ignition loss should exceed the quantity of organic matter, since it includes the combined water of the inorganic colloidal material and part of the sulphur and chlorine. The Florida peat has an ignition loss of 95.6 per cent. In this case the ignition loss must be nearly the correct value for organic matter, since very little water can be combined with inorganic material. Here the combustion value for organic matter, though obviously a little high, is more nearly correct than the hydrogen peroxide value. The ignition loss of the mosslike peat, 96.5 per cent, shows that, in

this case, the hydrogen peroxide value is the more accurate. More-over, the fact that there is very little carbon in the undecomposed residues from this peat is another indication that the hydrogen peroxide value is very nearly correct. The muck subsoil has an ignition loss of 13.24 per cent, which is probably the correct value for organic matter, since the muck appears to be a mixture of quartz sand and organic matter, little or no water being combined with the organic material. The combustion value here is certainly too high, and the peroxide value is too low. In the case of the Hagerstown loam, the difference between the percentage of organic matter lost through ignition (9.52 per cent) and the percentage obtained by com-bustion (9.22 per cent) is only 0.30 per cent. This soil would contain ordinarily about 2.50 per cent of water combined with the inorganic colloidal material, since it contains 25 per cent of colloidal matter which averages 10 per cent of combined water. The ignition loss indicated in this case is 7.02 per cent of organic matter. The com-bustion value seems to be too high, therefore, by a difference of 2.20 per cent, and the peroxide value too low by a difference of 2.10 per cent. The ignition losses would seem to indicate that in one determi-nation the combustion value is the more accurate, that in another determination the peroxide value is the more accurate, and that in two cases there is no choice between the two methods.

The correct value for organic matter is probably very close to the sum of the organic matter obtained by the hydrogen peroxide method, and the carbon in the residue; but it is probably slightly greater than this sum, since the carbonaceous matter in the residue is presumably not pure carbon. In any case this sum would represent the minimum quantity of organic matter present and would be only slightly less than the true value, as the quantity of carbon in the residue is small. The variation of the hydrogen peroxide values and the combustion values from this assumed correct value are shown in columns 5 and 7, respectively, of Table 7.

It is to be noted that in 11 cases there is no choice between the combustion and hydrogen peroxide methods, the values given by these methods differing by 0.16 or less from the assumed correct values. In 8 instances the combustion values are closer than the peroxide values, the average variation of the peroxide values and the maximum variation being 0.55 per cent, and 1.29 per cent, respec-tively, of the weight of the soil. In 7 determinations the peroxide results are nearer the assumed correct values. The combustion values in these 7 cases differ by an average of 3.04 per cent, and show a maximum deviation of 10.25 per cent.

It is apparent that the peroxide method gives about as accurate results, although usually low, as the combustion method. In the case of somewhat unusual soil materials also the results may be considerably more accurate.

ACCURACY WITH WHICH CONVENTIONAL FACTOR OF 0.471 APPLIES TO ORGANIC MATTER DESTROYED BY HYDROGEN PEROXIDE

It has just been shown that some of the combustion values for organic matter are more or less incorrect. Obviously the reason for this is that the factor used for calculating the carbon dioxide to organic matter does not correctly represent the composition of the organic matter of certain soils.

The use of the factor, 0.471, was first proposed by Wolff (*15*), in 1864. He found that there was, on the average, 58 per cent of carbon in the humus extracted from soils and in artificial humus preparations. To get the weight of organic matter, therefore, he recommended multiplying the quantity of carbon dioxide evolved on oxidation by the factor, 0.471. This factor has been used for the determination of organic matter in soils ever since.

It is clear that a factor derived from the average composition of soil organic matter may be quite inaccurate when applied to any particular soil, although it may correctly represent the average composition of the organic matter of a number of soils. From a large number of subsequent determinations, however, it is apparent that there may be considerably less than 58 per cent of carbon in the average soil organic matter. For instance, the average percentage of carbon in the humus extract of 40 soils, as given by Eggertz (*3*), Cameron and Breazeale (*1*), and Fraps and Hamner (*4*) is 45.6. This evidence is not conclusive, since the humus extract is only a part of the organic matter, and its composition may not be representative of all the organic matter present. Snyder (*12*) and Read and Ridgell (*8*) determined the carbon in the entire organic matter in 81 samples of soil. The average percentage of carbon in these 81 samples was 49.4.

The above averages indicate that the factor, 0.471, may be too low for the average soil. Further evidence regarding the accuracy of this factor is evidently desirable. The data in Table 7 bear on this point. By the use of these data, it is possible to calculate the percentage of carbon in most of the organic matter in each of the 26 samples examined. The hydrogen peroxide destroyed almost all the organic matter in many of these soil materials. The weight of the organic matter destroyed in each case is known, and the percentage of carbon in the organic matter may be calculated by subtracting the carbon in the residue from the total carbon, as determined by combustion, and dividing the remainder by the organic matter decomposed by hydrogen peroxide. The proportions of carbon calculated in this way are given in column 7 of Table 8.

The figures given in Table 8 show that the percentage of carbon in the organic matter decomposed by hydrogen peroxide varies considerably, ranging from 37.5 to 70.1, with an average of 54.8 per cent. Of the 26 samples tested, 5 samples (Nos. 15, 19, 20, 21, and 22) show determinations of slight dependability on account of the low organic content; and one soil, the Hagerstown loam, gives an abnormally high carbon percentage, on account of the artificial addition of charcoal. The remaining 20 samples cover a wide range of soils, including as they do, 2 peats, 2 mucks, and 3 soil colloids. Excluding Nos. 8, 15, 19, 20, 21, and 22, for the reasons noted above, the average carbon content for the remaining 20 is 56.3 per cent, with a minimum of 49.8 per cent and a maximum of 61.9 per cent.

When all the results of digestion by hydrogen peroxide are considered, the average percentage of carbon is considerably higher than the averages reported by investigators subsequent to Wolff. In the selected list, the average is still higher, being fairly close to the 58 per cent required to make Wolff's (*15*) conventional factor applicable.

TABLE 8.—*Quantity of carbon in the organic matter of various types of soil destroyed by hydrogen peroxide*

Sample No.	Type of soil and colloid	Depth at which soil sample was taken	Quantity of organic matter as determined by decomposition with hydrogen peroxide	Quantity of combustible matter calculated as carbon in residue from H_2O_2 treatment	Quantity of carbon in total organic matter of soil [1]	Quantity of carbon in organic matter destroyed by hydrogen peroxide
		Inches	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Amito loam	0–12	2.43	0.48	66.5	59.9
2	____do____	12–36	1.70	.37	64.4	57.0
3	Blakeley loam	0–12	2.43	.23	63.2	59.9
4	Carrington loam	0–12	3.59	.30	59.6	56.2
5	Carrington loam colloid	0–12	10.19	.41	63.3	61.9
6	Cukre clay	0–5	9.05	.67	57.0	53.9
7	____do____	5–60	2.25	.28	59.4	54.1
8	Hagerstown loam	0	4.92	1.90	78.4	70.1
9	Houston black clay	0–12	3.64	.60	57.5	50.5
10	Lenox stony loam	0–7	4.87	.50	55.3	50.6
11	Mosslike peat	0	95.57	.28	51.8	51.9
12	Muck	0–6	23.16	1.29	58.0	55.7
13	____do____	6–12	12.61	.60	61.8	60.1
14	Ontario loam	6–12	3.05	.06	57.2	56.5
15	Orangeburg fine sandy loam	10–36	.36	.06	62.1	56.4
16	Orangeburg fine sandy loam colloid	0–10	3.63	.38	61.9	58.0
17	Peat	46–70	90.67	1.45	61.3	60.8
18	Palmar clay	0–14	6.87	.27	59.8	58.3
19	Do	14–60	1.06	.07	44.6	41.0
20	Do	60–72	.85	.10	44.5	37.5
21	Do	72–96	.63	.06	57.4	53.4
22	Do	96–120	.51	.04	46.4	42.1
23	Ranger loam	0–10	1.90	.32	58.5	51.3
24	Sharkey clay	0–4	4.01	.44	54.6	49.8
25	Velasquez loam	12–32	13.17	.73	62.1	60.8
26	Wabash silt loam colloid	0–15	4.69	.50	61.9	57.9
	Average, all samples				58.6	54.8
	Average, excluding Nos. 8, 15, 19, 20, 21, and 22				59.7	56.3

[1] It is assumed that the organic matter not decomposed by H_2O_2 is carbon.

Although the preceding calculation applies to a larger part of the total organic matter than the calculations of Wolff, Eggertz, and others who have worked on humus extracts, it does not apply completely to the total organic matter, since a small quantity is not decomposed by hydrogen peroxide. There is some evidence that the organic matter not decomposed by hydrogen peroxide is higher in carbon than that which is decomposed. If this is so, it would raise the percentage of carbon in the total organic matter, in each case to a figure slightly higher than that expressed by the figure in column 6, Table 8. On the assumption that the organic matter in the residues is all carbon, the percentage of carbon in the entire organic matter of the soil was calculated. The results are given in column 7 of Table 8. The average carbon content for the 20 selected soils is 59.7 per cent. Doubtless the true average percentage for the total organic matter lies at some point between 56.3 and 59.7 per cent of carbon. It can thus be seen that the conventional factor, 0.471, corresponding to 58 per cent carbon, applies very well to the average organic matter in the soil types used in this study.

In individual cases the application of the factor leads to comparatively large errors, the maximum being 10 per cent. However, these errors occurred in soils so very high in organic matter that the combustion method is not ordinarily applied to them. The Hagerstown loam is an exception, because the large quantity of charcoal present in it is responsible for considerable error. From these results, it

seems reasonable to conclude that occasionally large errors may result from the use of the conventional factor. In general, however, this factor seems to represent the composition of organic matter rather accurately.

LIMITATIONS AND ADVANTAGES OF THE HYDROGEN PEROXIDE METHOD

From the point of view of manipulation the hydrogen peroxide method is satisfactory. The agreement of duplicates is good. The basic idea is simple, though fully as much care must be given to details of procedure as in the combustion method. The hydrogen peroxide method offers no advantages so far as rapidity of determination is concerned, over the combustion method, except when a large number of analyses are to be made.

The hydrogen peroxide method is not applicable to soils which contain more than 2 or 3 per cent of calcium carbonate or more than a trace of manganese dioxide or chromium sesquioxide. Soils containing chromium sesquioxide are rare; and soils containing more than a trace of manganese dioxide are, as a rule, confined to the tropical or subtropical latitudes.

As a means of determining the total organic matter in soils, the hydrogen peroxide method shows little, if any, inferiority to the combustion method. The peroxide method always gives low results; but the errors are small in most cases. The combustion method, on the other hand, sometimes gives too high or too low results, and occasionally the errors are large.

Although hydrogen peroxide does not completely decompose the organic matter in the soil, there is no good evidence that it determines any clearly defined kind of organic matter. The so-called "humus" is entirely destroyed; but since peat, crude fiber, and other cellulose materials are likewise decomposed by hydrogen peroxide, the method obviously can not be taken as a means for determining the humus in soils. Neither can the part of the soil which is not decomposed by peroxide be regarded as containing all the charcoal originally present, for under some conditions considerable charcoal is decomposed.

It is presumed that the carbonaceous matter remaining in the residues from the peroxide treatment is inert and hard to oxidize by biological processes. Probably this unattacked carbonaceous matter is not so important in soil fertility as the organic matter which is attacked by hydrogen peroxide. On the other hand, the peroxide method obviously can not be used to determine the readily available organic matter.

For routine analysis, the hydrogen peroxide method offers no advantage over the combustion method. However, in investigations where an accurate determination of the organic matter is necessary, as in the determination of the water which is combined with the inorganic material of soils, a combination of the hydrogen peroxide and combustion methods offers distinct advantages. If the carbon in the residue from the hydrogen peroxide digestion were determined by combustion and then added to the organic matter determined by hydrogen peroxide, the sum would be a more accurate figure for the total organic matter than the value obtained by any of the present methods.

SUMMARY

A report is given of the possibility of determining the organic matter in soils by the loss in weight caused by digestion with hydrogen peroxide, and a method for this determination is described.

Several forms of organic matter, including cellulose, humus, and lignite, are almost completely decomposed by hydrogen peroxide in the presence of soil. Graphite is unattacked and charcoal and coal are partially decomposed.

Determinations made by the hydrogen peroxide method on a variety of soils and colloids containing from 0.42 to 95.85 per cent of organic matter show that practically all the organic matter is decomposed in some soils, and that in other soils considerable organic matter is unattacked.

The nature of the carbonaceous matter resisting decomposition by hydrogen peroxide is uncertain. In some soils, charcoal forms a major part of the undecomposed organic matter, but other organic matter may also be present.

Although the hydrogen peroxide method gives low results, it is, on the whole, about as accurate as the combustion method, and may be considerably more accurate in special cases.

The method gives some indication concerning the percentage of carbon in soil organic matter. In the 26 samples analyzed, the organic matter averaged about 58 per cent carbon. This is the proportion of carbon required by the conventional factor, 0.471, used for calculating the carbon dioxide evolved by combustion to organic matter. Individual cases show considerable variation from this average of 58 per cent.

The hydrogen peroxide method is not applicable to soils high in calcium carbonate, manganese dioxide, or chromium sesquioxide.

Apparently the hydrogen peroxide method does not determine any clearly defined kind of soil organic matter. It decomposes more than the so-called "humus," and is therefore not a method to be used in determining the humified organic matter in the soil.

For routine analysis, the hydrogen peroxide method offers no advantages over the combustion methods. It should, however, prove useful in investigations, since this method, when it is supplemented by a combustion determination of carbon in undecomposed residues, should give the most accurate value for soil organic matter.

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DIPLODIA EAR-ROT DISEASE OF CORN[1]

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INTRODUCTION

The most common and destructive ear-rot disease of corn is caused by the fungus *Diplodia zeae* (Schw.) Lév. This disease is found in almost every locality in which corn is grown.

The first definite description of this fungus was given by Heald, Wilcox, and Pool (7),[2] and a more careful and exhaustive study of the disease was made later by Burrill and Barrett (3) working in Illinois. They showed that the ears were invaded either from the butt or the tip and that ears in the milk stage were most susceptible to infection; that the stalks were relatively immune to attack; and that the fruiting bodies of the fungus were produced freely on old stalks. Burrill and Barrett showed further that the spores readily survived the winter and were blown for considerable distances by the wind during the following spring. The two last-named writers estimated that 90 per cent of the ear-rot losses in Illinois were caused by this one disease.

Less than a decade ago *Diplodia zeae* was universally regarded as distinctly an ear-rot problem. Since the advent of the corn root-rot, stalk-rot, and ear-rot investigations, however, this fungus has been rather generally referred to in combination with several Fusaria as a causal agent for corn ear rot, stalk rot, and root rot. Thus in the report of the Ohio Agricultural Experiment Station for 1921 (16) appears the statement that "during the past three years careful investigation has been made for the purpose of determining the predominating cause of the corn root-rot disease in Ohio. This has been found to be the fungus *Diplodia zeae*, more generally known as a cause of corn dry rot or mold. This disease is present generally throughout the State, yet is most serious in the western and southwestern sections." Durrell (4) and Durrell and Porter (5) report that this fungus attacks the ears, shanks, and joints of the corn plant freely, but that it does not cause the roots to decay nor, under Iowa conditions, is it active in attacking the seedlings. They state further that following infection of the seed the stand obtained from this seed is considerably reduced numerically and the plants are weakened. Holbert, Burlison, Koehler, Woodworth, and Dungan (8), discussed *Diplodia zeae* as a cause of root rot, ear rot, and seedling blight of corn. They found that planting diseased seed gave a reduced stand and that the surviving plants made an irregular growth. Corn grown from infected seed was found to have more leaning stalks and stalks on the ground, but not more broken stalks.

Koehler, Dungan, and Holbert (10) state that "infection of seed corn with this organism [*Diplodia zeae*] results in a greater reduction

in yield than infection with any other organisms herein reported [*Fusarium moniliforme Cephalosporium acremonium*, and *Gibberella saubinctii*]."

At the present time *Diplodia zeae* is referred to not merely as cause of ear rot but also as a factor in the root, stalk, and ear rot situation. It is said to cause losses immediately as the result of molding ears, and in the long run, also, through the infection of seed and the consequent reduction in crop yield.

The present investigation was begun in 1921 in Ohio, where it was pursued two years. The writer continued the work in New York during the years 1923, 1924, and 1925. While many phases of the problem have been touched upon incidentally, the problem of seed infection and the effect of this infection on the germination, development, yield, and incidence of ear rot have been the major objectives.

RESULTS OF EXPERIMENTS

Field observations in Ohio in 1921 showed that the ear rot caused by *Diplodia zeae* was most prevalent in the western part of the State where corn was a major crop. A survey of the seed situation in this section was made during the winter with the idea of discovering the prevalence of Diplodia infection. The results of testing 2,900 ears for 82 farmers located in six counties showed 24.2 per cent of the ears infected—an exceptionally high average. Most of these infected seed ears did not show marked external signs of disease. This led to the supposition that they had been infected late in the summer. Through the following experiment more definite information was obtained as to the times during which natural infection took place in the fall and late summer of 1921.

A large field of corn growing on the farm of the Ohio Agricultural Experiment Station, Columbus, Ohio, was visited at intervals, beginning August 31, when the ears were in the milk stage. Each time approximately 60 ears were removed at random, taken to the laboratory, and placed in racks to dry. The corn in this field was cut September 27; hence all selections after that date were made from the the shocks. The results of a careful test of these various lots of ears made early in the winter are shown in Table 1.

TABLE 1.—*Occurrence of natural ear-rot infection on corn picked at different periods ranging from August 31 to December 1, 1921, on farm of Ohio Agricultural Experiment Station*

Date ears were picked	Number of ears picked	Percentage of ears infected	Percentage of kernels per infected ear		
			Healthy	Diseased	Dead
Aug. 31	60	6. 6	34. 38	31. 25	34. 37
Sept. 20	51	9. 8	32. 50	20. 00	47. 50
Oct. 10	64	18. 7	46. 87	31. 26	21. 87
Oct. 28	67	19. 1	45. 63	23. 62	30. 75
Dec. 1	60	40. 0	29. 25	38. 50	32. 25

These data show that some infection had already occurred by August 31 while the corn was still in the milk stage, and that the percentage of ears infected increased rapidly up to December 1, the date of last sampling. Especially notable is the increase in the percentage of infected ears which took place during November while the corn was standing in the shock. This increase, however, does not necessarily prove the occurrence of new infections during this period. It is quite possible that conditions within the shock were favorable for the growth of the fungus, and infections that were present on the ear, shank, or stalk at the time the corn was cut were, under these favorable conditions, transferred to the corn ears. In any case, however, the importance of selecting and drying seed corn early in the fall is emphasized. Further proof that the rot fungus readily invades matured ears is available as the result of the following inoculation experiments.

Two lots of 25 ears each were inoculated August 31, when the corn was in the milk stage, and two more on September 20, when the corn was almost ripe.

The inoculation was done by wounding each ear in the shank and pressing into each wound a bit of clover stem on which the fungus had been cultured. The results of the inoculations are shown in Table 2.

TABLE 2.—*Results of early and late infection with ear rot of two lots of corn of 25 ears each on August 31 and September 20, 1921. Ohio Agricultural Experiment Station*

Date inoculated	Percentage of ears infected	Percentage of kernels per infected ear		
		Healthy	Diseased	Dead
Aug. 31	100.0	0	7.5	92.5
Sept. 20	84.0	53.2	34.3	12.5

These ears were tested late in the year after the corn was perfectly dry. The lots inoculated while the corn was in the milk stage were badly molded, light in weight, with cobs showing evident decay. The ears were such as would be thrown out at husking time as worthless. The late-inoculated ears, on the other hand, showed no cob rot or surface growth of the fungus. The cobs of these ears looked clean and reasonably free from discoloration. The results of these tests indicate that the badly molded ears found at husking time represent early infections, while the less severely diseased ears found in seed corn represent late infections that were soon checked by the drying out of the corn.

The environmental factors favoring infection and rotting of the ears have not been determined under controlled conditions. Observations, however, indicate that abundant moisture during the early fall is highly favorable. After ear infection has once occurred the loss of moisture incidental to the drying out of the ears is commonly the factor which brings to a close the activity of the fungus. Corn-

rot fungus in ears stored at temperatures favoring fungus activity may, after the ears are completely air-dry, become inactive. Since no moisture determinations were made in the experiments previously referred to, the following data from Kiesselbach and Ratcliff (*9*) are given to indicate the approximate moisture content of corn that is thoroughly air-dried:

	Percentage moisture in embryo	Percentage moisture in endosperm
New corn (field selected):		
Before fully dry	31. 1	24. 1
When air-dry	10. 5	12. 9
Old corn:		
When air-dry	7. 91	7. 6
Embedded 24 hours in moist soil	21. 37	15. 62
Embedded 48 hours in moist soil	36. 81	20. 83

As would be expected, the activity of the fungus in diseased ears very gradually decreases as the ears become drier, until the point is reached when further advances can not be detected by the germination test.

The following records of a test made in the fall of 1921 illustrate the gradual manner in which fungal growth ceases as the infected ears of corn become dry. Mature ears of dent corn harvested September 27 were placed in a warm attic and allowed to remain there until October 7, at which time they appeared to be dry. Tests were made and records taken on that date and again one month later. In making the two tests, kernels side by side were removed from the ears. The records of the two tests are shown in Table 3.

TABLE 3.—*Progress of Diplodia infection as influenced by drying of ears, Ohio Agricultural Experimental Station, 1921*

Date of test	Percentage of kernels per ear		
	Healthy	Diseased	Dead
Oct. 7	56. 7	43. 3	0
Nov. 4	53. 2	34. 3	12. 5

It will be observed that in those ears which were rapidly approaching the air-dried condition the rate of fungal advance was very slow. All the ears used in the test were inoculated September 20, so that during the 2½ weeks immediately following inoculation 34.3 per cent of the kernels were invaded, while during the next 4 weeks the percentage of infected kernels was increased by only 3.5 per cent. It is apparent that early field selection of seed corn and immediate drying are very effective in reducing the amount of Diplodia infection.

HOW CORN-EAR INFECTION OCCURS

The spores of the fungus causing corn-ear rot are carried through the air. The tip of the ear surrounded by the partly dead and dried silk remains would seem to offer a favorable point of entrance for these spores, especially since the corn-ear worm, which attacks at this point, might logically be supposed to open the way for ear-mold infection. Durrell (*4*), however, states that under Iowa conditions in 1921 the

majority of infections began at the butts of the ears. The writer's observations over a period of years are fully in accord with this conclusion. Thus, for example, a record was kept of some 1,700 ears comprising 170 exhibits at a corn show. The highest percentage of infection in any exhibit was 70 and the lowest 0. The average for the entire show was 13.3 per cent. Owing to the fact that these ears had been selected especially for exhibit this average was much lower than the seed corn average in the district for the year. Of the entire number of diseased ears, 89.9 per cent were infected at the butt, 2.7 per cent at the tip, and 7.4 per cent at points between the butt and the tip. These figures, coupled with other observations, indicate that this fungus has little need for the assistance of the ear worm. The fact that seed ears are invaded by a number of fungi in addition to Diplodia is well known. Manns and Adams (*11*) have attempted to determine the relative prevalence of these organisms. Their data show that two Fusaria were very prevalent everywhere. These organisms, *Gibberella saubinetii* and *Fusarium moniliforme*, were very common in the seed corn mentioned above. It seemed worth while, therefore, to determine the immediate damage caused by the Fusaria as compared to Diplodia. It was not thought advisable to attempt to distinguish between the two species of Fusaria. Ears infected with Diplodia averaged 8.8 per cent of the kernels dead, while ears infected with the Fusarium averaged 2.3 per cent of the kernels dead. Hence Diplodia infection is much more likely to result in the death of the kernel than Fusaria infection. This is explained by the fact that the Diplodia attacks the embryo by preference, while the Fusaria invade the starchy portions of the kernels.

DIPLODIA ZEAE AS A CAUSE OF SEEDLING BLIGHT

This fungus is common in seed corn. Many kernels in infected ears are invaded but not killed. When these diseased but living kernels are planted they soon absorb sufficient moisture to permit fungal activity. It should be borne in mind, however, that whereas in the fall moisture is the factor that limits the development of the ear-rot phase of the disease, temperature appears as the factor limiting the development of the seedling blight phase in the spring. Thus, if diseased seed is planted in warm soil, the fungus quickly grows out from the kernel and attacks the mesocotyl of the seedling. Since the mesocotyl tissues are very susceptible, decay progresses rapidly as far as the primary node. Beginning at the primary node, the tissues are resistant; hence the advance of the rot is sharply checked at this point. The secondary roots thrown out at the primary node are not attacked. If the diseased seedling lives until these are established, it is safe. Under temperature conditions favorable for the disease some seedlings are killed before the secondary roots develop.

The relationship of temperature to the development of seedling blight was tested as follows: Four tanks were filled with water. Two of them were kept at a temperature of 15° to 18° C. by permitting cold water to trickle into them. The other two were maintained at a temperature of 29° to 31° C. by heating them. Two 3-gallon crocks filled with sterilized soil were placed in each tank. Four similar crocks were placed on a bench in the greenhouse, where the temperature ranged from 21° to 24° C. Sowings of diseased and healthy ker-

nels were made at uniform depths in all of these crocks and the resulting plants were allowed to grow for three weeks. At the end of this period all plants were carefully washed free from the soil and the results tabulated. Table 4 is a record of the results of several experiments of the character described.

TABLE 4.—*Effect of soil temperature on the development of seedling root rot*

Kind of seed planted	Temperature (°C.)	Number of kernels sown	Percentage of seed not sprouted	Percentage of seedlings with roots—		
				Healthy	Severely rotted	Slightly rotted
Healthy (control)	15–18	40	2.5	97.5	0	0
Do	21–24	40	2.5	97.5	0	0
Do	29–31	40	7.5	92.5	0	0
100 per cent diseased	15–18	24	58.3	37 5	0	4.2
Do	21–24	24	37.5	8 3	50.0	4.2
Do	29–31	24	16.7	25.0	45 8	12.5
Diseased and healthy, half and half	15–18	32	21.8	65.7	9 4	3.1
Do	21–24	32	40.7	21 8	34.4	3.1
Do	29–31	32	21.9	25.0	31.2	21.9

Table 4 shows that the coolest temperature was unfavorable and the two warmer soil temperatures favorable for the development of the seedling root rot. It seems safe to conclude that there will be little rotting at temperatures below 20° C.

Repeated plantings of diseased seed in the field have resulted in very slight development of seedling root rot. This is apparently accounted for by the fact that temperatures of field soil during the seedling stage are generally below 20° C. A consistent feature of the field plantings has been the poor stand resulting from diseased seed. Thus healthy seed germinating on test 100 per cent would give a field stand of not less than 95 per cent, while diseased seed germinating on test 60 per cent would be likely to give a field stand of 40 to 45 per cent. Table 4 shows that the diseased seed produced a poor stand at the coolest temperature. Holbert, Burlison, et al. (*8*), state that Diplodia-diseased seed produced a poor stand in cool weather and a much better stand in warm weather. The fact that numbers of kernels, seriously attacked and weakened by the Diplodia fungus, lack the strength to push through the soil is the reason assigned for the poor stand with cool temperatures.

RELATION OF DIPLODIA SEED INFECTION TO LOSS OF STAND DURING THE GROWING SEASON

Up to this point the discussion has considered (1) the problem of infection of seed ears with Diplodia in the fall, the period of activity of the fungus in the ears, and the relation of the fungus to moisture content, and (2) the effect of this seed-borne infection on germination and stand in the field during the seedling stage. There remains for consideration the effect of corn ear rot on stand later in the season.

The literature on corn root rot contains numerous references to weak and stunted plants and also to the death of plants. During the course of the experiments covered by this discussion counts were made as soon as the plants were well up in the spring and again late

in the summer when the ears were maturing. The results for each year were the same, which the 1924 data as recorded in Table 5 will serve to illustrate.

TABLE 5.—*Effect of seed infection on stand of corn throughout summer, at New York (Geneva) Agricultural Experiment Station, 1924*

Plot No.	Stand on June 25 from—		Stand on November 25 from—	
	Healthy seed	Diseased seed	Healthy seed	Diseased seed
	Stalks	*Stalks*	*Stalks*	*Stalks*
1	55	55	52	51
2	54	54	51	51
3	56	56	56	54
4	58	58	50	58
5	56	56	55	53
6	55	55	54	52
7	58	58	56	57
8	58	58	55	58
9	60	60	60	36
10	58	59	57	57
Total loss in stand			22	22

That the loss in stand happened to total exactly the same for the diseased and healthy seed was, of course, merely a coincidence. In other tests the loss from healthy seed sometimes exceeded the loss from diseased seed, and vice versa. Never, however, has there been any indication that the planting of Diplodia-infected seed resulted in any loss of stand during the summer months.

The experiments established beyond reasonable doubt that Diplodia seed infection does not destroy plants except in the very early seedling stages, and that the plants growing from diseased seeds which survive the early attack of the fungus are as likely to mature as plants from healthy seeds. Another fact apparently well established is that the secondary root system is not susceptible to attack. Durrell (4) placed large quantities of inoculum directly under a number of growing plants without effect. The writer has repeatedly planted healthy seed and scattered rice culture of the fungus thickly in the hills about the seed without any perceptible effect. As a more severe test of the resistance of plants grown from healthy seed to corn root rot, healthy seeds were sown in crocks of sterilized soil which had been inoculated with the fungus in pure culture. One series of these crocks was placed in the greenhouse. The roots of plants in the diseased soil showed slight decay at a number of points. This experiment was made in 1922. In another experiment, made in 1923, healthy seed was grown under field conditions. In this experiment the roots from diseased and healthy soil appeared equally bright and clean. Under field conditions, therefore, the fungus does not cause a root rot. Valleau, Karraker, and Johnson (15) report that numerous isolations from decaying corn roots failed to yield *Diplodia zeae* in any case.

RELATION OF SEED INFECTION TO EAR-ROT INFECTION

The question of the possible relationship of seed-borne infection to later outbreaks of ear rot is one of great importance. Does the planting of infected seed result in a crop of infected ears? Smith

and Hedges (*13*) have reported that the fungus may spread to the stalk from the roots and have advanced the theory that ear infection may result in this manner. For four years, however, the writer has planted diseased and healthy seed side by side without securing any consistent variations in the percentages of infected ears. If ear infection ever results from the spread of the fungus from the roots, this occurs so seldom as to be a negligible factor. This was likewise the conclusion of Van der Bijl (*1*).

There still remains, however, the possibility of a definite relation between seed infection and later development of ear rot. The ability of the fungus to grow as far up as the base of the stalk has been proved. This would be sufficient to bring it to the surface of the ground. It is possible that fruiting bodies and spores produced by the fungus at this stage may be distributed by wind and rain to the ears. On this assumption, plantings of diseased and healthy seed made side by side would be exposed equally to infection. This may be the possibility that Tryon (*14*) expresses as follows: "This 'tainted' seed will not, should it germinate, as only happens when the infection is light, directly give rise to a plant in whose system, consequently, the disease will develop; but, failing to do so, it may, when placed under circumstances favorable to growth of the fungus, yield the Diplodia spores that may prove a source of eventually infecting many other maize plants that otherwise would remain healthy; and 'dead seed' will, with utmost likelihood, do so."

To determine the possible relationship between seed infection by corn root rot and the later development of ear rot, plantings of diseased and healthy seed were made in plots well separated from one another during the three years 1923, 1924, and 1925. The ears from each planting were harvested separately and carefully tested during the winter. In no case was there evidence that the sowing of diseased seed had any influence on the quantity of ear rot in the resulting crop. The corn grown from diseased seed was quite as free from ear rot as the corn grown from healthy seed.

The low incidence of ear rot in the corn grown from these plantings of diseased and healthy seed is worthy of note. In the 1925 experiment only 2 out of 960 ears tested showed Diplodia infection. Corn had not been grown for three years or more previously in the soils used for these tests, and the fodder had been removed in the fall from the few cornfields in the near vicinity. The failure of ear-rot infection under these conditions points to the efficiency of rotation and sanitation control measures. In their original work Burrill and Barrett (*3*) emphasized the value of rotation and removal of the fodder from old fields. They showed that the spores were distributed freely and for considerable distances by the wind and that the old stalks from the previous year's crop left scattered over the surface of the ground were infection sources.

EFFECT OF SEED INFECTION ON THE VIGOR OF STALK GROWTH AND ON THE YIELD OF EAR CORN

In a test made in 1921 diseased and healthy ears were carefully selected. This corn was shelled separately, giving in one case seed 100 per cent healthy and in the other seed of which portions were either dead, diseased but living, or healthy. This second lot will be

referred to as the diseased seed. Sowings were made in adjacent rows. The results of this test are recorded in Table 6.

TABLE 6.—*Effect of seed infection on corn yield per plant at Ohio Agricultural Experiment Station, 1921*

Kind of seed	Number of kernels planted	Number of stalks obtained	Total yield in pounds	Yield per stalk in pounds
Healthy	200	186	98.7	0.531
Diseased	200	108	59.4	.550

There was no visible difference in the vigor of growth of the corn produced by the two lots of seed. The slight difference in yield favoring the diseased seed is fully accounted for by the difference in stand.

In 1923 a large number of ears were tested very carefully and diseased and healthy lots selected. In several tests the healthy ears showed no evidence of fungal infection. The diseased ears showed infection with Diplodia only. These two lots of ears were shelled separately. The healthy seed was then divided into halves. One half was inoculated with pure cultures of the fungus grown on rice; the other half was held untreated. These three lots of seed—healthy, inoculated, and diseased—were then used to plant two series of plots. Series 1 was sown on May 8 and series 2 on June 5. Each plot consisted of a single row of 10 hills, 3 plants to the hill, and the hills 3 feet apart. Sufficient seed was sown in each hill to permit later thinning to a uniform stand. The planting and thinning were both done by the writer, and in every case great care was taken to insure absolute impartiality in removing the plants.

These plots were kept under close observation the entire summer. No differences in vigor of growth were observed. The plants from diseased seed, from healthy seed, and from healthy seed inoculated, grew equally well.

In the fall yields from the plots located in the two outside rows of the experimental block were disregarded. The corn in other plots, however, was husked and the yields of ear corn recorded, as shown in Table 7.

TABLE 7.—*Effect of seed infection on corn yields at New York (Geneva) Agricultural Experiment Station, 1923*

Kind of seed	Number of stalks		Number of bearing stalks		Weight of ears		Weight of corn per bearing stalk		Average weight per bearing stalk
	First planting	Second planting	First planting	Second planting	First planting	Second planting	First planting	Second planting	
					Ounces	Ounces	Ounces	Ounces	Ounces
Healthy	29	30	28	29	246	357	8.8	12.3	} 10.67
Do	29	30	28	29	298	320	10.6	11.0	
Healthy inoculated	30	30	27	27	288	318	10.7	11.8	} 10.9
Do	30	30	27	25	321	234	11.9	9.4	
Diseased	30	30	30	28	337	284	11.2	10.1	} 10.4
Do	30	30	29	28	282	298	9.7	10.6	

From the data presented in Table 7, it may be seen that the yields per bearing stalk for diseased, healthy, and healthy inoculated seed are very close together, with the averages slightly favoring the healthy seed as compared to the diseased seed.

This experiment was repeated on a larger scale in 1924. Preparations for the experiment were begun in the fall of 1923, when two lots of bright, clean ears were selected from each of two ear-to-row plantings. A number of these ears were inoculated as follows: Cavities were scooped out at the butt ends and the pure cultures of Diplodia fungus placed therein. These inoculated ears were then stood on moist cotton in Mason jars. They were held under these conditions one week and then removed and allowed to dry. A germination test after they were fairly dry showed that in most of these inoculated ears the fungus had penetrated about half the length of the cob. By this procedure it was possible to secure diseased and healthy seed in sufficient quantity and from the same ear strain. Any ears which showed upon testing chance infections of other fungi were discarded. As in the previous experiments, healthy seed was also inoculated by mixing with it just prior to planting pure cultures of the fungus. The land chosen for this test was exceptionally level and uniform. It had not been cropped to corn for several years.

The seed was sown on May 28. On June 25 all plots were thinned to an even stand. The plots were kept under constant observation throughout the season, but no differences in growth could be detected. In November the corn was harvested and the ear weights shown in Table 8 were recorded.

TABLE 8.—*Relation of seed infection to total corn yield and yield per bearing stalk at New York (Geneva) Agricultural Experiment Station, 1924*

Kind of seed planted	Plot No.	Number of stalks matured	Number of barren stalks	Total weight of ears	Weight of ears per bearing stalk
				Ounces	*Ounces*
Diseased	1	52	1	334	6.5
Do	2	51	6	266	5.9
Do	3	56	2	340	6.3
Do	4	50	8	256	6.1
Do	5	55	7	315	6.6
Average		52.8	4.8		6.28
Healthy, inoculated	1	54	6	299	6.2
Do	2	53	14	224	5.7
Do	3	56	11	274	6.1
Do	4	55	19	222	6.2
Do	5	56	19	225	6.1
Average		54.8	13.8		6.06
Healthy seed	1	55	7	282	5.9
Do	2	51	15	233	6.5
Do	3	54	13	249	6.1
Do	4	53	9	275	6.2
Do	5	53	17	208	5.8
Average		53.2	12.2		6.10

The figures for yield per bearing stalk fall very close together, with the results slightly in favor of the diseased seed. There was an increase of barren stalks in the healthy inoculated plantings as

compared with the diseased plantings. These differences appear to have been associated with imperceptible soil differences, since the buffer plantings, all sown with the same seed, showed the same variations.

The three-year comparison of diseased and healthy seed made in the experiments already discussed, as well as the observations of others, fail to show that Diplodia-infected seed noticeably influenced the vigor of growth or reduces the yield. It should be emphazied, however, that this refers not to plantings of 100 per cent diseased seed as compared with plantings of healthy seed, but to plantings of the usual composite obtained from diseased ears, which includes both healthy and infected kernels.

RELATION OF SEED INFECTION TO INCIDENCE OF LEANING OR BROKEN, BARREN, OR PREMATURELY DEAD STALKS, OR BROKEN SHANKS

During the course of these experiments notes were made for the purpose of discovering if possible any correlation between seed infection and the incidence of leaning or broken stalks, barren stalks, prematurely dead stalks, or broken shanks. No such correlation was found. This is quite in line with Durrell's observations (*4*). Koehler, Dungan and Holbert (*10*), on the other hand, report more leaning stalks from Diplodia-infected seed than from healthy seed. The differences they discovered, however, were small.

DISCUSSION OF RESULTS

Many of the results obtained in these experiments merely corroborate the findings of previous investigators. It was discovered that the fungus invades ears with the greatest ease, usually entering at the butt end. Ears which are infected as the corn approached maturity do not suffer conspicuous injury, and hence are likely to be selected as seed. Under favorable environmental conditions seedling root rot resulted from plantings of infected seed. In the field, however, soil conditions at corn-planting time were such that this difficulty was not encountered. The only immediately noticeable result from the planting of diseased seed was the poor stand.

The possible relationship of seed infection to ear infection later was carefully investigated. An argument often advanced against the planting of diseased seed corn is that this practice will result in an increased percentage of unsound ears. This theory is based on the supposition that there is a correlation between seed infection and the later occurrence of ear rot. These experiments covered by this discussion have failed to demonstrate a connection between Diplodia seed infection and Diplodia ear rot. On the contrary, the evidence leads to the conclusion that the farmer who plants diseased seed is not increasing his chances of suffering loss from this form of ear rot at least.

The question of the effect of seed infection on the later growth of the crop and on the yield is also very important. Durrell and Porter (*5*) state that "in addition to the reduction in stand, it was very noticeable that the rows planted with Diplodia dry rot infected seed had a high percentage of weak, stunted plants, which either died or were so weakened that they reduced the yield." Their

figures, however, do not show any very serious influence of this sort. Thus in their 1922 tests the stand reduction from the planting of the diseased seed was 8.6 per cent and the reduction in the yield 7.7 per cent. In 1923 the stand reduction was 12.1 per cent and the yield reduction 12.2 per cent. Koehler, Dungan, and Holbert (*10*) report a stand decrease of 36.3 per cent and a yield decrease of 32.4 per cent from sowing diseased seed. These authors state that "infection of seed corn with this organism results in a greater reduction in yield than infection with any other organisms herein reported." The other organisms referred to by these authors were *Fusarium moniliforme*, *Cephalosporium acremonium*, and *Gibberella saubinetii*. In all of these experiments, however, the reduction in yield is attributed to two factors—(1) the decreased stand and (2) the weakened condition of the plants. The effect of decreased stand on the yield, however, need not be allowed to intrude, since Kiesselbach and Ratcliff (*9*) have worked this out to a mathematical certainty. The real question involved is how seriously is the vigor of stand reduced as the result of sowing diseased seed.

In considering this question, attention must be called to the experimental procedure followed in the Illinois tests. In this work a number of diseased ears was selected, from each of which all healthy seed and all dead seed were discarded, leaving only the kernels that were infected but alive. The diseased seed which would be planted by a farmer, however, would be quite different. To begin with, it is not likely that more than 30 per cent of the farmer's seed ears would be infected. The average infected seed ear would test about one-eighth dead, three-eighths alive but diseased, and four-eighths healthy kernels. Adding to the 70 per cent of the farmer's healthy seed the four-eighths, or 15 per cent, portion of his diseased ears that tested healthy gives a total of 85 per cent of his shelled seed corn that is actually healthy. Of the diseased 15 per cent remaining, one-eighth, or 3.75 per cent, is dead. This leaves a total of only 11.25 per cent that is diseased but living and hence capable of producing the weakened stalks.

Actually, many of these diseased seeds when sown in the field will not produce any plants. Assuming that every diseased seed will grow and produce a stalk, the ratio of stalks from healthy seed to that of stalks from diseased seed will be more than 8 to 1. Under actual field conditions, therefore, every plant produced by a Diplodia-infected seed would be surrounded by plants from healthy seed, whereas in the Illinois test artificial restrictions were resorted ⌄ to in order to insure as nearly as possible a stand of plants from diseased seed only. It is easy to see how under these conditions a measurable reduction in yield owing to reduced plant vigor could result. Under natural conditions it is not at all difficult to understand how slight weakness on the part of one-ninth of the stand would be largely compensated for by the other eight-ninths. It is on this basis that the results obtained in the experiments in which there was no measurable decrease in yield from the planting of seed from diseased ears, when this seed was sown thick enough to give a 100 per cent stand, are explained.

In these experiments the great majority of stalks in the diseased seed plots were produced by normal, healthy kernels. This is accounted for by the fact that many of the diseased kernels were

dead at the time of planting and many others lacked the strength to reach the soil surface. Futhermore, the reduction in yield obtained from planting only diseased seed need not be ascribed to a pathological activity continuing through the life of the plant, but may be fully accounted for on the basis of the immediate destructive activity of the fungus in the seed. The effect of this activity would be to weaken the seed, and there are good reasons for believing that, in the case of corn, weakening of the seed will reduce the yield. Thus Brown (*2*) has shown that mechanically multilated seed when planted gives a poor stand and weak-growing plants which yield less than the controls grown from perfect seed. He found that the reduction in yield was proportionate to the amount of mutilation. Schmidt (*12*) and others, working with sweet corn, have shown marked yield differences favoring large as compared to small seed. In the experiments here detailed 100 per cent Diplodia seed infection, has been produced through smearing the shelled seed with pure cultures of the fungus just prior to planting. This method of infecting seed eliminates the weakening effect on the seed due to fungal invasion. Seed so inoculated has repeatedly grown and yielded as well as the uninoculated healthy seed.

In this connection it is interesting to note Edgerton's conclusion (*6*) concerning *Cephalosporium acremonium* and *Fusarium moniliforme*. As the result of several years of field testing, he states that diseased seed corn gives poor stands, but that the plants from poor ears seemingly produce as much corn as the plants from good ears, and that no material differences in yield result if equal stands are obtained.

Any attempt to list the Diplodia ear-rot disease as a serious seed-borne trouble must take into consideration that the important diseases included in this class possess characteristics as follows:

First. Grain smuts, of which loose smut of wheat is a good example, cause a twofold loss. These diseases destroy the heads or ears of grain crops, thus reducing the value of the crops and in some cases reducing the quality of the crop as well. They infect the maturing seed, also, thus carrying their destruction into the next year. The relation of seed infection to head or ear destruction, and hence to seed infection and the infection of the crop of the subsequent season, is clearly drawn.

Second. Numerous vegetable crop diseases, such as black rot and black leg of the crucifers, blight and anthracnose of beans, may pass the winter in a very few seeds and yet be present in sufficient quantity to cause 100 per cent crop infection the following year. This is accounted for by the phenomenal ability of the organisms causing these diseases to multiply and spread from plant to plant during the growing season.

Diplodia when present as seed infection does not spread to the stalks and destroy the ears, as in the case with the smuts, nor does it serve as the original source from which the disease spreads from plant to plant, as is the case with vegetable crop diseases. It is the writer's opinion that Burrill and Barrett (*3*) covered the important phases of the Diplodia situation rather completely when they proved this fungus to be the cause of a widely destructive ear-

rot disease and demonstrated its ability to overwinter freely on old corn refuse and to spread from thence to the ears the following season.

SUMMARY

The fungus *Diplodia zeae* is the cause of a destructive corn ear rot. Infections, in the great majority of cases, begin at the butt, and the fungus apparently grows into the cob by way of the shank.

Under ordinary field conditions the ears are susceptible to infection almost from the time they are formed until they are mature; to cause extensive molding, however, fungal invasion must occur while the ears are immature.

The fungus remains active within the infected ears until they have become thoroughly dry.

Ears infected late in the season are not visibly molded, and hence are often selected for seed. When planted, seed from such ears gives a poor stand and a correspondingly reduced yield as compared to seed from healthy ears. In the experiments with which this discussion is concerned however, no differences in yield resulted if the seed from diseased ears was sown thick enough to compensate for the reduced germination.

Comparative plantings of diseased and healthy seed repeated over a three-year period showed no increase in ear rot due to sowing diseased seed.

Diseased and healthy seed sown on land not cropped to corn for three years or more and well removed from fields containing old stalks and remains of previous season crops have both produced corn almost 100 per cent free from Diplodia ear-rot infection.

Where Diplodia infection is anticipated, early removal of seed ears will avoid a large part of the infection. Prompt drying will check the spread of the fungus in cases where ear infection has already occurred.

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MEASURES OF PERSISTENCY OF LACTATION [1]

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INTRODUCTION

In agricultural colleges it is frequently taught that cows differ markedly in persistency of lactation, or the degree with which the milk flow is maintained as the stage of lactation advances after calving. The dairy breeds are considered superior to the beef breeds and unimproved cattle in this particular; and as between the individual cows of any one dairy breed there are differences in persistency.

Similar remarks may be made with reference to yearly yield. In this case there is abundant quantitative data to substantiate the teaching. Many determinations of the annual milk and fat yields of cows have been made, which give for each individual the year's production in the form of a definite figure. This is the important figure which measures the worth of the cow from a cost-accounting standpoint. From a biological standpoint we know that the yearly production is the resultant of a complex of forces or factors. It is desirable for the purpose of biological analysis to break up the total production into its component parts, so far as possible.

It seems logical to regard the yield of milk for any definite period of time as the result of the rate of milk secretion during that period. Milk secretion is initiated by the bearing of young, and the mammary gland reaches a maximum potential capacity for milk secretion periodically or recurrently with the close of gestation. From a biological standpoint, one may therefore deal with the rate of milk secretion during the period following calving. In the present state of development of the dairy cow, the function of lactation usually continues uninterruptedly for 10 months or even much longer, depending upon the recurrence of conception. Obviously, a suitable measure of the rate of milk secretion may give a measure of production which is independent of the length of time covered by the production record. Such a measure of the rate of milk secretion will indicate its initial value at calving and its change with advance in lactation. This latter is the characteristic commonly referred to as persistency of lactation, in the numerical evaluation of which the writer is especially concerned in this paper.

MEASURES OF PERSISTENCY

The earliest numerical measure of persistency which has come to the writer's attention is that of Sturtevant (8) [2] which expresses the decrease in milk yield each month as a percentage of the milk yield of the preceding month. This idea has been used by various investigators who followed Sturtevant. Carlyle and Woll (2) employed as a measure of persistency the ratio $100 \frac{a-b}{a}$ where a is the yield for a certain early week in the lactation period, and b is the yield for a certain late week in the lactation period. This principle has also

Journal of Agricultural Research,
Washington, D. C.

(373)

Vol. 34, No. 4
Feb 15, 1927
Key No. Ill. 23

been followed by other investigators. McCandlish et al. (*6*) have used the method of expressing each month's milk yield as a percentage of the first month's yield and comparing, graphically, the slopes of the curves thus obtained. This method does not give a numerical value to persistency.

There is evident a certain kind of relationship, between the three measures just described. They have been used as cited only in comparisons of groups of cows and not in detailed studies of individuals.

Obviously, what is needed is a numerical expression representing persistency for each individual cow so that the character may be studied quantitatively by statistical methods and brought into line with our knowledge of other quantitative characters. Sanders (*7*) has presented the first detailed study of this sort. As his basic measure of persistency he used the ratio of the lactation milk yield to the maximum day's yield. This measure is affected greatly by the length of the lactation, which in turn is affected by the time from calving to conception (service period). Sanders accordingly applies a correction for service period.

Turner (*9*) has recently proposed two measures of persistency. The first is essentially that used by Sturtevant and expresses the average ratio of each month's yield (except the first) to that of the preceding month's yield. The second is similar to that of Sanders and expresses the ratio of the twelve months' yield to the yield of the maximum month, from which ratio may be derived the supposedly corresponding ratio of the first method. The first method when applied to the irregular data of individual records is mathematically unsound as a measure of the general tendency of the record and may be dismissed from further consideration here.[3] The principle of the second method will be considered in some detail below.

The published records of the American Guernsey Cattle Club offer inviting material for the study of persistency. In studying the question of persistency in this laboratory, the writer has felt that it was desirable to obtain for each record under consideration an equation which would represent as accurately as possible the rate of milk secretion through the course of lactation undisturbed by pregnancy. Daily energy yield in terms of 4 per cent milk (*4*) is considered in preference to direct use of the raw data of milk or fat yield as published by calendar months.

Consideration was given first to the straight line equation

$$\frac{dy}{dt} = C - bt \ \text{------------------------(1)}$$

in which y represents yield; t, time from calving as origin; $\frac{dy}{dt}$, the rate of yield; C and b are constants, C representing the initial rate of yield and b the rate of *absolute* decrease in rate of yield. The constant b is used as a measure of persistency.

Equation (1) was abandoned in favor of the exponential equation

$$\frac{dy}{dt} = ae^{-\lambda t} \ \text{----------------------(2)}$$

in which y, t, and $\frac{dy}{dt}$ have the same meaning as in (1); a is a constant

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[3] It is not desired to imply that this method may not give a *practically* useful measure, but it tends to give too high values, especially when monthly yields are irregular.

representing the initial rate of yield; e is the base of natural loga rithms; and k is a constant representing the rate of decrease in the rate of yield as *proportional* to the rate of yield of the moment. The constant k is used as a measure of persistency.[4] Exponential expressions have been found of wide use in the description of both physical and biological phenomena. So far as the writer is aware, this type of equation was first used in description and interpretation of the lactation curve by Brody et al. (*1*).

DETERMINATION OF THE CONSTANTS

In a more or less preliminary investigation (*3*) of some of the Guernsey records the writer has used a special graphic method of determining the a and k constants of equation (2) for each record. If the observations fall in anything like a regular order, the method is satisfactory, but many of the records are so irregular that considerable uncertainty attaches to the graphic method. It is furthermore not adapted to routine clerical computation. For these reasons it is proposed to use the algebraic method of least squares in further study. The method gives theoretically the most probable values of the constants and is quite readily applied.

The various considerations involved in the use of equation (2) have been presented previously (*5*). It is sufficient here to say that the monthly yields of milk and fat are reduced by the formula $y_d = \dfrac{.4M + 15F}{L}$ where M is milk yield in pounds and F is fat yield in pounds for the month, and L is length of the month in days; y_d becomes thus the average daily energy yield in terms of 4 per cent milk in pounds for the month.[5] (One pound of 4 per cent milk = 331 large calories.) Considering the low values of k with which we shall have to deal, equation (2) is, for all practical purposes equal to

$$y_d = Ae^{-kt} \dots \dots \dots \dots \dots \dots (3)$$

in which $\dfrac{365 y_d}{12} = \dfrac{dy}{dt}$ and $\dfrac{365 A}{12} = a$, provided time is computed to the *middle* of the month from which the y_d value is obtained.

Equation (3) is applied to the observed data by taking logarithms on both sides to convert it to linear form. It takes its simplest form by the use of natural logarithms:

$$\log_e y_d = \log_e A - kt \dots \dots \dots \dots \dots (4)$$

Equation (4) is to be fitted to the 11 observations of the record by the method of least squares. Since the time origin (calving) is variable with respect to the middle of the month, it is convenient in computation to select a new time origin at the middle of the first full calendar month. Time in months from this origin we may designate T, and the time in months from calving to the middle of the first full calendar month, t'. Then $t = T + t'$. Let us designate the rate of yield at $T = 0$ as A', whence $A = A'e^{kt'}$ and $\log_e A = \log_e A' + kt'$. Equation (4) is thus equivalent to

$$\log_e y_d = \log_e A' - kT \dots \dots \dots \dots \dots (5)$$

[4] The difference between this measure and that of Sturtevant is that the rate of decrease is computed as occurring continuously instead of monthly. The "per cent of persistency" used by Turner corresponds to $100e^{-k}$.

It should be noted that the value of a is a hypothetical quantity representing theoretical potential capacity at calving. It would perhaps be better to deal with the lactation curve following one-half or one month after calving—that is, with $ae^{-.5k}$ or ae^{-k} instead of a—thus dealing more closely with actual yields. A very simple change in equations (7)-(17) would accomplish this end.

[5] Another feature of this reduction is that it gives values lying, with few exceptions, between 10 and 100, a distinct advantage mechanically for slide rule computation.

The normal equations for the least squares determination of A' and k of (5) for 11 observations at $T=0, 1, 2, ---10$ are:

$$11 \log_e A' - 55k = \Sigma \log_e y_d$$
$$55 \log_e A' - 385k = \Sigma \, T \log_e y_d$$

Solving:

$$55 \log_e A' - 275k = 5\Sigma \log_e y_d$$
$$55 \log_e A' - 385k = \Sigma \, T \log_e y_d$$
$$110k = 5\Sigma \log_e y_d - s \, T \log {}_e y_d$$
$$10k = 5\Sigma \frac{\log_e y_d}{11} - s \, T \frac{\log_e y_d}{11}$$
$$11 \log_e A' - 55k = \Sigma \log_e y_d$$
$$\log_e A' = \Sigma \frac{\log_e y_d}{11} + 5k$$

and hence,

$$\log_e A = \Sigma \frac{\log_e y_d}{11} + 5k + kt'$$

It is clear that it is desirable to have the values $\frac{\log_e y_d}{11}$, which we may designate by v. The value v is readily computed from the original record by the use of a specially graduated slide rule as described in Figure 1. The formulae for k and A become:
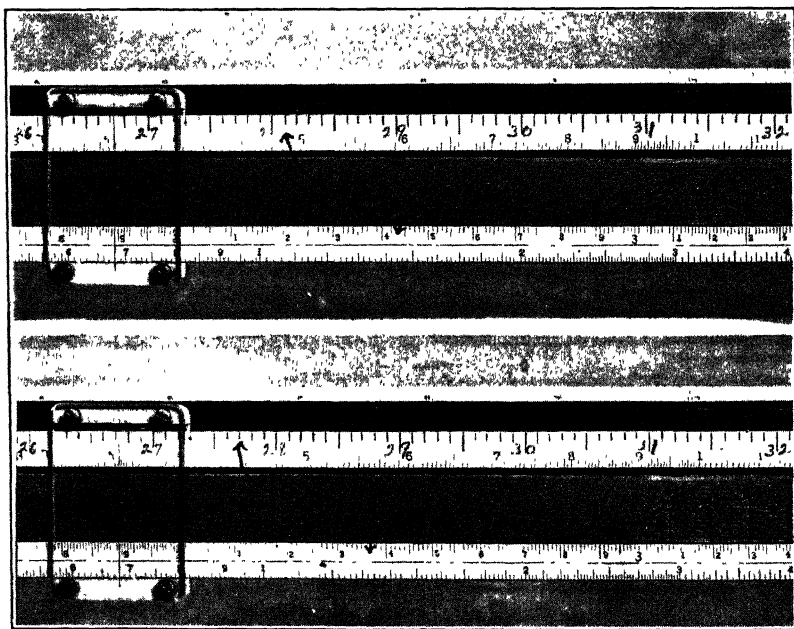
FIG. 1.—Portion of a slide rule adapted to the computation of the monthly values of v.

Example: The month record shows a 28-day month; 308 pounds of milk; 8.82 per cent fat.
    Solution: Set 28 of the L scale of the slide on 308 of the M scale; set the runner on 8.82 on the f scale of slide; under the runner on the v scale read $v=0.267$ (+).
    One may also read under the runner on the y_d scale, $y_d=19.0$ (−). The M and y_d scales are one and the same. Another L scale is provided at the opposite end of the slide. The f scale extends from 2.0 to 9.0. The example is chosen to condense the cut rather than as being typical.

(Formulae for use of
11 observations and
natural logarithms)

$$k = .1\,(5\Sigma v - s\,Tv) \quad\text{------------------} (6)$$

$$A = \log_e^{-1}[\Sigma v + k(5 + t')] \quad\text{------------} (7)$$

TABLE 1.—*Determination of the constants of the lactation curve by the method of least squares*

GUERNSEY A. R. No. 10233

Full calendar month	$T=$ time	$L=$ length of month	$M=$ milk	$f=$ fat	$F=$ fat	$y_d=$ $\dfrac{.4\,M+15\,F}{L}$	$u=$ $\log_{10} y_d$	$v=$ $\dfrac{\log_e y_d}{11}$
	Months	*Days*	*Pounds*	*Per cent*	*Pounds*	*Pounds*		
First	0	31	1,264 5	4.34	54.88	42.9	1.6325	0.342
Second	1	31	1,181.9	4.36	51.53	40.2	1.6042	.336
Third	2	30	1,179.6	4 40	51.90	41.7	1.6201	.339
Fourth	3	31	1,236.1	4.60	56.86	43.5	1.6385	.343
Fifth	4	30	1,181.6	4.47	52.82	42.2	1.6253	.340
Sixth	5	31	1,212.0	4.51	54.66	42.1	1.6243	.340
Seventh	6	31	1,205.4	4.75	57.26	43 3	1.6365	.343
Eighth	7	28	1,077.8	5 05	54.43	44.6	1.6493	.345
Ninth	8	31	1,115.8	4.81	53.67	40.4	1.6064	.336
Tenth	9	30	1,109.1	4.78	53.01	41.3	1.6160	.338
Eleventh	10	31	1,113.5	5 45	60.69	43 7	1.6405	.344

Σy_d, Σu, and Σv --- 465.9   17.8936   3 746
$\Sigma T y_d$, ΣTu, and ΣTv ------------------------------------ 2,337.3   89.5469   18.746
$\Sigma(T+1)y_d$, $\Sigma(T+1)u$, and $\Sigma(T+1)v$ --------------------------- 2,803.2   107.4405   22.492
Rate of decrease--- −.07   −.002   −.002
Initial rate of yield ($T= -1.4$)-------------------------------------- 41.9   41 9   41.9

GUERNSEY A. R. NO. 10372 [a]

First	0	31	1,609.4	4.56	73.39	56.3	1.7505	0.366
Second	1	29	1,244.1	4.96	61.71	49.1	1.6911	.354
Third	2	31	1,144.8	4.55	52.09	40.0	1.6021	.335
Fourth	3	30	1,022.9	4.52	46.24	36.8	1.5658	.328
Fifth	4	31	892.0	4.98	44.42	33.0	1.5185	.318
Sixth	5	30	856.8	4.57	30.02	23.8	1.3766	.288
Seventh	6	31	427.2	4.56	19.48	14.9	1.1732	.246
Eighth	7	31	319.9	5.19	16.60	12.2	1.0864	.227
Ninth	8	30	309.8	4.59	14.22	11.2	1.0492	.220
Tenth	9	31	288.9	5.40	15.60	11.3	1.0531	.220
Eleventh	10	30	223 7	4.67	10.45	8.2	.9138	.191

Σy_d, Σu, and Σv --- 296.8   14.7803   3.093
$\Sigma T y_d$, ΣTu, and ΣTv ------------------------------------ 938.6   64.2032   13.435
$\Sigma(T+1)y_d$, $\Sigma(T+1)u$, and $\Sigma(T+1)r$ --------------------------- 1,235.4   78.9835   16.528
Rate of decrease--- 4.96   .203   203
Initial rate of yield ($T= -1.0$)-------------------------------------- 56.7   74.5   74.5

[a] Note that No. 10372 would be dry at 11.4 months after calving ($T=10.4$) by equation (1); by equation (2) she would be milking at the rate of 7.3 pounds of 4 per cent milk per day at that time.

   Table 1, which shows the 11 full calendar-month records of two cows, illustrates the process of determining the constants of the lactation curve as just described. The data covering milk and fat yields and fat percentage are taken directly from the published record.

   The values of v are computed as described in Figure 1 and recorded in column v. Σv is simply the sum of the v's and ΣTv the sum of the products Tv. $\Sigma (T+1)v$ is carried out to check the work. $\Sigma (T+1)$ $v = \Sigma v + \Sigma Tv$. Σv and ΣTv are readily obtained on a suitable computing machine.

   For No. 10233, $t' = 1.4$ and for No. 10372, $t' = 1.0$. The k constant is derived from equation (6) and the A constant from equation (7)

and a table of natural logarithms. Having given the record itself, the only figures necessary to record are those given in the v column of Table 1. The whole operation thus becomes engagingly simple.

It is, of course, unnecessary to compute A if the measure of persistency is all that is desired. But it appears (3) that for equitable comparisons of the k's it is necessary to know the values of the A's for the purpose of correction.

Where conception occurs early in the record, pregnancy may introduce a disturbing factor in the later observations. The decrease in rate of yield due to pregnancy is not large and may be allowed for as follows: If at the end of the last month used the calf is carried less than 183 days no correction need be made. Where the calf is carried 183 to 213 days as above, correct the last observation by multiplying by 1.02, that is, *add* 0.002 to the last observed v. Where the calf is carried 214 to 244 days, correct the next to last observation as above, and correct the last observation by the factor 1.06, that is, *add* 0.005 to the last v. These corrections are based on the effect of pregnancy as previously given. (*5*)

Where pregnancy is more than 244 days advanced as above, it may be desirable to work with nine observations or with seven, as the case requires. Corrections to the last one or two v's are made as indicated above.

The normal equations using nine observations are:

$$9 \log_e A' - 36k = \Sigma \log_e y_d$$

$$36 \log_e A' - 204k = \Sigma T \log_e y_d$$

From which we derive:

(Formulae for use of $\quad k = .183(4\Sigma v - \Sigma\ Tv)$ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _(8)
nine observations
and natural loga- $\quad A = \log_e^{-1}[1.222\Sigma v + k(4 + t'')]$ _ _ _ _ _ _ _ _ _ _(9)
rithms.)

The normal equations using seven observations are:

$$7 \log_e A' - 21k = \Sigma \log_e y_d$$

$$21 \log_e A' - 91k = \Sigma T \log_e y_d$$

From which we derive:

(Formulae for use of $\quad k = .393(3\Sigma v - \Sigma Tv)$ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _(10.)
seven observations
and natural loga- $\quad A = \log_e^{-1}[1.571\Sigma v + k(3 + t')]$ _ _ _ _ _ _ _ _(11)
rithms.)

The majority (about 80 per cent) of the Guernsey records may be handled with equations (6) and (7). The value v is not particularly useful in connection with equations (8), (9), (10), and (11), but it still affords as easy a solution as may be had; and its retention is justified in order to utilize the already established device of Figure 1.

If it is desired to work directly with the milk or fat yields, or if the equation is to be fitted to only a small number of records, it may be preferable to use common logarithms instead of natural logarithms.

The data necessary to record and the final results are given as examples in column u of Table 1. The following formulae in which $u = \log_{10} y_d$, give results identical with those above.[6] To correct for pregnancy add 0.0086 and 0.0253 to u in the same manner as 0.002 and 0.005, respectively, to v above.

(Formulae for use of 11 observations and common logarithms.)

$$k = .0209 \ (5\Sigma u - \Sigma Tu) \ \text{---------------------(12)}$$

$$A = \log_{10}^{-1}[.09091\Sigma u + .4343k \ (5 + t')] \ \text{----(13)}$$

(Formulae for use of 9 observations and common logarithms.)

$$k = .0384 \ (4\Sigma u - \Sigma Tu) \ \text{----------------(14)}$$

$$A = \log_{10}^{-1} [.11111\Sigma u + .4343k \ (4 + t')] \text{----(15)}$$

(Formulae for use of observations and common logarithms.)

$$k = .0822 \ (3\Sigma u - \Sigma Tu) \ \text{-----------------(16)}$$

$$A = \log_{10}^{-1} [.14286\Sigma u + .4343k \ (3 + t')] \ \text{----(17)}$$

Another method of determining the k constant deserves consideration. From equation (2) the rate of yield at one month after calving is ae^{-k} and the yield for 12 months is $a \dfrac{1 - e^{-12k}}{k}$. Accordingly, the ratio of the former to the latter is $\dfrac{ae^{-k}}{a \dfrac{1 - e^{-12k}}{k}} = \dfrac{k}{e^k - e^{-11k}}$.

It is apparent that the $a's$ cancel and any given value of k results in a certain definite ratio. Conversely, if the ratio is given, the value of k may be computed. We have given the 12 months' yield. If we let the yield of the first full calendar month represent the rate of yield one month after the record starts (assuming that the first of that month is one-half month after the beginning of the record), we may determine the required ratio.

The theoretical ratios corresponding to various values of k are as follows:

k	−0.04	−0.03	−0.02	−0.01	0	0.01	0.02	0.03	0.04	0.05
Ratio a	2,222	2,345	2,473	2,605	2,740	2,879	3,020	3,166	3,315	3,466
Ratio b	2,312	2,417	2,523	2,631	2,740	2,850	2,960	3,072	3,185	3,297

k	0.06	0.07	0.08	0.09	0.10	0.11	0.12	0 13	0.14	0.15
Ratio a	3,619	3,776	3,934	4,095	4,257	4,421	4,585	4,751	4,919	5,084
Ratio b	3,408	3 521	3 632	3,743	3,852	3,960	4,067	4,172	4,275	4,376

k	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25
Ratio a	5,252	5,420	5,587	5,754	5,921	6,086	6,250	6,414	6,575	6,734
Ratio b	4,476	4,572	4,666	4,757	4,847	4,933	5,015	5,097	5,173	5,247

a $\dfrac{12}{365} \times \dfrac{k}{e^k - e - 11k} \times 10^6$. This ratio is based on the use of the first full calendar month's yield per day, assuming the middle of this month to be one month after the beginning of the record.

b $\dfrac{12}{365} \times \dfrac{k}{e^{2k} - e - 10k} \times 10^6$. This ratio is based on the use of the second full calendar month's yield per day, assuming the middle of this month to be two months after the beginning of the record.

It should be observed that the relation between the k's and the ratios is not linear. Persistency as measured by the k's is therefore not directly comparable with persistency as measured by the ratios.

[6] It is of course easier to deal directly with the calendar month milk or fat yields, in which case let $u = \log_{10} M$ and $\log_{10} F$, respectively, and $A = a$, in equations (12) to (17). As Turner has pointed out, it is necessary to distinguish between persistency in *milk* yield and persistency in *fat* yield—the decrease in rate of fat yield being ordinarily less rapid than the decrease in rate of milk yield. Persistency in *energy* yield is intermediate (5, fig. 1), and takes account of the variability between individual cows in the change of fat percentage with advance in lactation.

The estimation of k by this method is very rapidly carried out by the use of an appropriately graduated slide rule. The slide carries the L and f scales as shown in Figure 1 and in addition, a regular logarithmic scale matching the logarithmic M scale. A k scale replaces the r scale of Figure 1. The computation requires two settings of the slide and one setting of the runner, the value of k being read opposite the unity graduation of the logarithmic scale of the slide.

By entirely similar methods the ratio of the second month to the year may be used in place of the ratio of the first month. The ratios for the second month are also given in the tabulation immediately preceding

The value of A is given by the formula $A = \log_e^{-1} (\log_e y_d + t')$ where y_d is determined from the first month and t' is time in months from calving to the middle of the first month. The estimations assume that pregnancy is not a factor.

The constants of the straight line equation (1) are determined thus

$$b = .0091 (5\Sigma y_d - \Sigma T y_d) \tag{18}$$

$$C = .0909 \Sigma y_d + b(5 + t') \tag{19}$$

where 11 observations are used and pregnancy is not a factor to be considered. Examples of the application are given in the y_d column of Table 1.

COMPARISON OF PERSISTENCY MEASURES

From the Guernsey records previously used (*3*) in which k of equation (2) was determined graphically, 100 were selected at random, except that only records were taken in which not more than 30 days elapsed from calving to the first of the first full month. For the purpose of comparison the value of k in these 100 records has been computed by least squares and the two ratio methods; also the value of b in equation (1) has been computed by (18). In none of these records was pregnancy far enough advanced to require a correction.

The values of k and b have been correlated with the values of k by least squares, giving the constants set forth in Table 2. The mean values of k as determined graphically and by the ratio methods do not differ significantly from the mean as determined by the method of least squares. A high degree of variability as measured by the coefficient of variability is evident throughout.

TABLE 2.—*Statistical constants of persistency of lactation as determined by different methods*

Measure of persistency	Mean	Standard deviation	Coefficient of variability	Relation to k by least squares— coefficient of correlation
k by least squares	0.0382±0.0020	0.0300±0.0141	78.52	
k graphically	.0398± .0021	.0311± .0148	77.94	0.956±0.006
k from first month and year	.0377± .0021	.0304± .0148	80.53	.866± .017
k from second month and year	.0415± .0024	.0350± .0170	84.34	.902± .013
b by least squares	1.118 ± .058	.857 ± .041	76.66	.921± .010

The mean value of b is, of course, much greater than that of k, but it is of interest to note that the coefficient of variability of b is nearly the same as that of k. The coefficients of correlation show that taking k by the method of least squares as the standard, k by the graphic method gives the next best result; b by least squares next; followed by the second month ratio. The first month ratio is least closely related.

DISCUSSION

. If we accept the monthly observations as each of equal reliability and accept also equation (2) as a proper description of the lactation curve, then k as determined by the method of least squares should be the most probable persistency value for any given record. The k values obtained graphically correspond reasonably well; $r = 0.956$. While the graphic method possesses some advantage in speed, the algebraic method has the advantage of being an exact process and adapted to routine computation.

In the graphic treatment 8.5 per cent of the records were rejected because of extreme irregularities (3). The fact that the algebraic process leads to a perfectly definite final result for these records as well as the others does not make that final result of perfectly definite significance. For that matter and from the same point of view, the definite figure of the 12 months' yield may be lacking in definite significance. The value of k determined by least squares would seem to afford a measure of persistency at least as definite in significance as the year's yield itself.

The estimation of k by the ratio method has a great advantage in point of speed, and it was largely to test its reliability that the present comparisons were made. Apparently it is better to use the second month rather than the first, although it is doubtful if the difference is significant. The reliability of this rapid method of estimating k may be tested by correlating the k's as determined from the first and second months. The correlation surface given in Table 3 shows $r = 0.792$. There would seem to be some question, therefore, as to the trustworthiness of this method of estimating k.[7] This is unfortunate, since the method is a short and easy one.

The ratio method used by Sanders (7) and Turner (9), namely $\dfrac{\text{total yield}}{\text{maximum rate of yield}}$, fails to distinguish the ascending lactation curve from the more usual descending curve. Inasmuch as about 5 per cent of the Guernsey records show an ascending lactation curve, it is evident that this feature should be distinguished. Furthermore, as will be sufficiently evident from the ratios given above, the use of a ratio of this kind as a consistent measure of persistency must take the short time yield at a definite stage of the longer time period.

The use of equation (1) gives a measure of persistency which is apparently in close keeping with that of the least squares k of equation (2), $r = 0.921$. So far as the writer can see, there is no a priori reason why b of (1) should not be used as a measure of persistency. Nevertheless, it appears more reasonable to express the rate of decrease as proportional to the level of yield.

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[7] The present treatment takes account of the length of the calendar month. Failure so to do would, of course, add to the uncertainty of the result.

TABLE 3.—*Correlation of the k's computed from the ratio of the first month's yield to the year's yield and the ratio of the second month's yield to the year's yield* [a]

$k\times10^3$ from second month and year-class mid-points	$k\times10^3$ from first month and year-class mid-points														Total
	−25	−15	−5	5	15	25	35	45	55	65	75	85	95	105	
−25				1											1
−15			1	1	1										3
−5	1	1	2	1		1		1							7
5	1		1	3		1		1							7
15	1		1	1	1	1		1	1						7
25		1	1	2	3	6	2	1							16
35			1	2	2	5	2			1					13
45					1	2	1	4	3			1			12
55							1	1	4	1	1		1		9
65								1	1	1	3	1			7
75								1	2	2				1	6
85							1		1			1			3
95												1			1
105													2		2
115										1			2	1	4
125															0
135															0
145													1		1
155														1	1
Total	3	2	6	9	8	11	14	15	13	5	3	4	4	3	100

[a] $r=0.792\pm0.025$ See Table 2 for other constants

The lactation curve is distinctly nonlinear, but only slightly so, [cf. numerous figures (*1, 5*)]. Indirect statistical evidence that such is the case is afforded by the correlation between rate of yield and yield for the year. By (1) it is evident from the simple geometry of the equation that the area from $t=0$ to $t=12$ would be entirely determined at $t=6$, regardless of the slope of the curve (granting that $C>12b$). We should accordingly expect the highest correlation between rate of yield and year's yield when the rate of yield is taken at $t=6$. The observed correlations show the highest value somewhat earlier than this. On the basis of the descending exponential curve, this is to be expected.

Another and much more important distinction between the two types of equations as a measure of persistency, may be brought out by an illustration. McCandlish et al. (*6, chart 2*) have presented graphically the lactation curves of purebred dairy cows and scrub cows in the Iowa Station herd. Fitting equations (1) and (2) to the monthly milk yields [8] by the method of least squares gives the following:

	Purebred cows	Scrub cows
Equation (1),	$\dfrac{dy}{dt}=1055-70.9t$	$\dfrac{dy}{dt}=629-55.9t$
Equation (2),	$\dfrac{dy}{dt}=1169e^{-.114t}$	$\dfrac{dy}{dt}=848e^{-.213t}$
Rate of decrease by (1)	70.9	55.9
Rate of decrease by (2)	.114	.213

By the linear equation the scrub cows are considerably more persistent milkers; i. e., show a smaller rate of decrease than the pure-

breds. By the exponential equation the purebreds are nearly twice as persistent as the scrubs. Are the scrubs or the purebreds the more persistent?

Critical study of persistency and of the lactation curve in general has not yet been carried to a point that affords very complete knowledge of the subject. The available data should be utilized to the fullest extent for the purpose of obtaining a correct understanding of the characteristics, heritable and otherwise, of the lactation curve.

The official records as now published by breed organizations do not give any direct information as to persistency. In particular, the year's yield is an exceedingly poor index of persistency. Contrary to general opinion, the seven-day record early in the lactation far excells the year record in this respect. One object of securing a satisfactory numerical description of the lactation curve (not necessarily the easiest of computation) is the possibility that such a description offers of presenting the essential facts in condensed form and thus within the means of publication for individual records.

SUMMARY

Several methods for measuring persistency of lactation are reviewed. The measure preferred by the writer is the constant k in the equation $\frac{dy}{dt} = ae^{-kt}$, in which y is yield, t is time, and $\frac{dy}{dt}$ is the rate of yield. The constant k measures the rate of decrease in rate of yield as proportional to the rate of yield. It is thus an inverse measure of persistency. Formulae for fitting the equation by least squares are given in simplified form for the special cases of 7, 9, and 11 observed values.

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THE VITAMIN C CONTENT OF FRESH AND CANNED PEAR [1]

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INTRODUCTION

It has become a well-established fact that the water-soluble vitamin C is necessary not only as a protection from scurvy but also in normal metabolism and in the maintenance of the defense of the body against the attacks of infectious diseases. Since vitamin C is necessary in the adequate diet, it is expedient that a knowledge of foods which can be used to supply this factor be acquired. Little is known in regard to the antiscorbutic value of some of the more common fruits which are grown in this section of the country and which are abundant in the local markets. This investigation was made in order to determine whether or not pears which were grown locally could be used as a source of vitamin C, and therefore substituted for citrus fruits, which are purchased for their antiscorbutic value.

Data are not available on the vitamin C content of pear, but investigations have been conducted on other fruits which may be grown in this region. Kohman, Eddy, and Carlsson (7),[3] in their work with apples, indicate that vitamin C is destroyed by heat and oxidation in the processes of cooking and canning if no preliminary treatment has been given. They found that when raw apples were held in cold storage a marked deterioration occurred in the vitamin C content, although storage seemed to make no difference after the fruit was canned. Givens, McClugage, and Van Horne (2) found 10 gm. of raw apples to be a minimum protective dose against scurvy in guinea pigs. They reported that exposure to temperatures necessary for drying or canning caused a marked loss in the antiscorbutic factor. The minimum protective dose of raw peach was found by Kohman, Eddy, and Carlsson (8) to be 5 gm. daily. A daily dose of 20 gm. was necessary when the fruit was cooked in a covered kettle for 15 minutes. They found that the commercially canned peach had between four and five times as much vitamin C as fruit cooked in a covered kettle.

The present paper deals with the determination of the minimum of fresh pear required to prevent scurvy in guinea pigs. This determination was then used as a basis for estimating the loss in the antiscorbutic value which occurs in the pear when preserved according to the open-kettle and cold-pack methods of home canning.

METHODS

The vitamin C determinations were made in the usual manner on guinea pigs. The procedure of Sherman, LaMer, and Campbell (*12*) was closely followed. Cages, in which the animals were confined singly, were cleaned on alternate days and sterilized weekly. During the entire period the animals were weighed three times a week and all abnormal symptoms noted.

The basal diet recommended by the above-named authors is as follows:

	Per cent
Oats, sound whole grain, ground	5ᵃ
Skimmed milk powder, heated for two hours at 110° C	50
Butter fat, filtered and freshly prepared	10
Sodium chloride	1
	100

This diet was slightly modified by substituting cod-liver oil for 2 per cent of the butterfat.

Kieffer pears of sound quality and purchased on the local market in September were used. In February the supply of fresh pears was exhausted, and a new lot of California pears was purchased to finish the experiment. This product was fed raw until April, the animals showing the same growth as had been made while they were fed on the home-grown product. Some of the raw pears were kept in a fruit-storage room at a temperature of 40° F. They were wrapped separately in paper so as not to come in contact with each other, and every two weeks they were sorted and the spoiled ones removed.

The ripest of the Kieffer pears were canned according to the following methods of home preservation:

1. *The open-kettle method.*—The fruit was peeled, cored, and halved. In an open kettle were placed 430 gms. of raw pear, 100 gms. of sugar, and a minimum amount of distilled water. When the boiling point was reached, the fruit was allowed to boil for 15 minutes and immediately sealed in sterile pint jars.

2. *The cold-pack method.*—The fruit was prepared as in the open-kettle method. The same amounts of fruit and sugar were placed in sterilized jars, and enough distilled water was added to fill the jars. They were sealed and processed for 20 minutes after the temperature of the water surrounding them reached the boiling point. In the household the jars are sealed after the processing, but in this experiment the order was reversed to avoid possible loss of product.

With a known amount of fruit, sugar, and water in each can, it was possible to calculate, in terms of raw pear, the actual amount of canned pear fed. The amount of pear given to each animal was calculated on the basis of the amount that would have been fed to a 300-gm. guinea pig, as it was thought that by so doing more uniform results would be obtained. In feeding the canned pear it was found that the entire amount given each day was not always consumed, perhaps because of the sugar, in the pear. It was, necessary therefore, to calculate the amount wasted, which may have introduced an element of error into the results.

The raw pears were allowed to ripen in storage, and feeding was started in November. The animals were given 5, 10, 15, 20, 25, 30, or 35 gm. amounts of raw pear, or 10, 15, 20, 25, 30, or 35 gm. amounts of canned pear. Fifty-five animals, 53 males and 2 females, were fed. Negative control animals were given only the

basal diet in order to make sure that they received no vitamin C. Positive control animals were given the basal diet and 5 c. c. of tomato juice.

Autopsies were performed in all cases soon after death occurred. Bacteriological cultures were made to determine whether or not infections existed. A study of the gross microscopic pathology was made according to Hess (*5*). The histological method used was that suggested in the study made by Wolbach and Howe (*13*).

RESULTS

The symptomatology of scurvy which was found in the present experiment was similar to that described by Jackson and Moore (*6*), Cohen and Mendel (*1*), Hart, Steenbock, and Smith (*3*), Hess (*5*), and others. The first indication was lameness of the hind legs, followed by soreness and swelling in the joints and muscles and an
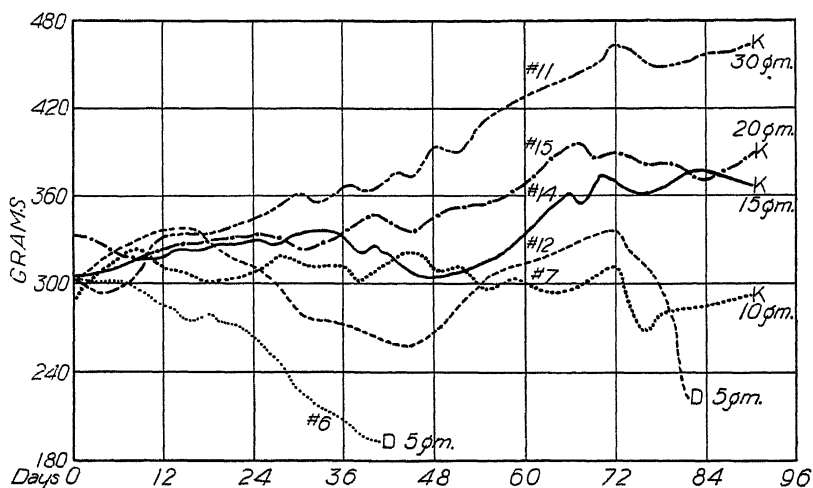
Fig. 1.—Growth curves of guinea pigs receiving from 5 to 30 gm. of raw pear daily. The growth curves of animals not surviving the 90-day experimentation period are marked "D"; those of animals killed at the expiration of the 90 days are marked "K"

increasing inactivity, which finally developed into an apparent paralysis of those parts. There was an increased flabbiness of the muscles and a general loss in body weight, resulting in emaciation. The autopsies showed hemorrhage in the muscles, particularly in the tissue surrounding the femur and the costochondral junctions. In some cases intestinal hemorrhage, preceded by bloody diarrhea, was noticed, while in others there was hematuria. Some congestion was found in the stomach and bladder, and in three cases an ulcerated condition in these organs. Contrary to the findings of Jackson and Moore (*6*), almost no hemorrhagic condition was observed in the gums. The teeth became yellow with the onset of the disease. Different degrees of looseness were found in the lower molars, and in some instances these could be removed easily with forceps. In no case were the incisors loose, and some animals had no loose teeth at all. The costochondral junctions, particularly of the middle ribs, were found to have the characteristic beading of scurvy. The spleen and lymph nodes were enlarged in two instances, a condition described by Jackson and Moore (*6*).

A study of the microscopic pathology was made according to Hess (*5*), only gross changes in the tissues being noted. The study of the costochondral junctions showed disorganization of the cells.

As is indicated in Table 1, two animals which were given 5 gm. amounts of raw pear did not survive the 90-day experimental period. Guinea pig 6, on 5 gm. of raw pear, died at the end of 40 days (fig. 1), and had only very slight symptoms of scurvy. There was a slight hemorrhage in the costochondral junction, some fatty degeneration of the liver, and slight congestion in the lungs. These symptoms might indicate reasons for death other than scurvy, although the bacterial cultures showed no pathogenic organisms. Guinea pig 12, on 5 gm. of raw pear, showed some hemorrhage in the stomach, but the other organs appeared normal. The bones were somewhat brittle, and the lymph nodes had become enlarged. The animals fed 10 gm. of raw pear gave no indications of scurvy during life, and the autopsies revealed no sign of a vitamin C deficiency. The only indication was a negligible loss in body weight. The 15 gm. amounts gave good protection to the animals, as is shown by the fact that in most cases there was some gain in body weight. The other animals on varying amounts of raw pear made varying gains in weight. (Table 1 and fig. 1.) The unexpected gain made by guinea pig 17 on 30 grams of raw pear may have been due not only to the large amount of fruit given but also to the fact that it was a larger animal at the beginning of the experiment.

TABLE 1.—*Protocols of experimental animals fed the basal diet and raw pear*

Animal No.	Amount of pear eaten daily	Initial weight of animal	Final weight of animal	Gain or loss in weight	Duration of experiment
	Gm.	Gm.	Gm.	Gm.	Days
6	5	302	187	−115	40
12	5	302	216	−86	81
7	10	308	290	−18	90
13	10	339	333	−6	90
9	15	295	281	−14	90
8	15	295	394	+99	90
14	15	303	363	+60	90
19	15	305	328	+23	90
15	20	332	383	+51	90
20	20	359	478	+119	90
10	25	308	334	+26	90
16	25	300	356	+56	90
11	30	303	458	+155	90
17	30	336	575	+239	90
18	35	305	437	+132	90

The average survival period for the animals fed open-kettle pear was 25.2 days. These animals were given large quantities of the canned fruit, but the entire amounts were not always eaten by every guinea pig. As is indicated in Table 2, it was found that 8.9 grams was the smallest amount and 19.1 grams was the largest amount of pear eaten daily. Table 2 also shows that characteristic scurvy lesions were found in the animals fed on open-kettle pear. The symbols x, xx, and xxx are used to denote increasingly marked findings.

TABLE 2.—*Protocols of experimental animals fed the basal diet and open-kettle pear*

Animal No.	Amount of pear eaten daily	Initial weight of animal	Final weight of animal	Loss in weight	Duration of experiment	Degree of malnutrition,[a] as indicated by—		
						Teeth	Bones and ribs	Hemorrhage
	Gm.	*Gm.*	*Gm.*	*Gm*	*Days*			
49	8. 9	326	187	139	32	xx[b]	xx	x
50	10. 9	320	214	106	27	xx	x	xx
52	12. 9	297	248	49	19	xx	x	xx
59	15. 6	297	214	83	15			x
57	17. 1	220	195	25	19	xx	x	xxx
53	17. 6	306	280	26	27	xx	xx	xx
58	18. 1	299	241	58	22		x	xx
54	18 3	304	295	9	24	x	x	xx
56	18. 3	320	173	147	30	xx	x	xxx
51	19. 0	305	176	129	38	xxx	xx	xx
60	19 1	304	265	39	25	x	xx	xx

[a] Determined by post-mortem examination.
[b] The symbols x, xx, and xxx, indicate the relative severity of the condition found.

Figure 2 shows the growth curves of the guinea pig eating the smallest amount and of the one eating approximately the largest amount daily, and it can readily be seen that the larger amount gives very little more protection than does the smaller amount. In fact, the animal eating the largest daily amount (No. 60) lived only 25 days, which is slightly below the average for the group. In no case did any animal fed open-kettle pear live more than 38 days—an interval only a little longer than the average expectation of life of the animals on the basal diet alone, which, according to Sherman, La Mer, and Campbell (*12*), is 26 to 34 days. The figures for this

FIG. 2.—Growth curves of two guinea pigs, one eating 8.9 gm. and the other 19 gm of open-kettle pear daily. The growth curves are marked "D," indicating that neither animal survived the full experimentation period of 90 days

series fall within those limits. Figure 3 shows the growth curves of three animals—one eating the smallest amount of cold-pack pear daily, one an approximately medium amount, and one the largest amount.

Number of grams of pear eaten daily	Average survival period, in days
5–9	29. 5
9–13	34. 4
13–17	38. 6
17–21	38.
21–25	[a] 50.

[a] One guinea pig.

In no case did an animal survive the experimental period and all the guinea pigs showed some symptoms of scurvy in the post-mortem examinations. The extent of these symptoms is indicated in Table 3. The autopsy findings showed that severe symptoms did not always accompany early death.

TABLE 3.—*Protocols of experimental animals fed basal diet and cold-pack pear*

Animal No.	Amount of pear eaten daily	Initial weight of animal	Final weight of animal	Loss in weight	Duration of experiment	Degree of malnutrition [a] as indicated by—		
						Teeth	Bones and ribs	Hemorrhage
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Days*			
42	6. 80	356	214	142	25	x \ [b]		
43	7. 18	358	210	148	32	x	x x x	x
31	8. 00	317	189	128	33	x x x	x x x	x x
44	8 89	363	227	136	28	\	x x	x x
46	9 87	346	185	161	37	x x x	x x	\ \
47	10 00	348	183	165	46	x \ x	x	\ \
37	10. 90	362	252	110	22	\	\ x	x
38	11. 36	388	210	178	33	\	x	\
41	12. 00	302	166	136	25	x x	x x	\ x
35	12. 00	357	194	163	37	x x x	x x	x x
48	12. 20	357	192	165	41	x x x	x x x	x x
25	13. 00	329	195	134	33	x x	x x	x x
26	14. 30	322	206	116	40		x x x	x x
24	14. 34	285	164	121	46		x	\ x
36	14. 60	320	193	127	44	x x	x x	\ x
27	15 60	356	223	133	30		x x x	\ x x
28	17 00	340	197	143	27		x x x	x x x
40	20. 40	377	219	158	49	x x x	x x	\
29	22 50	291	205	86	50		x x	\ \

[a] Determined by post-mortem examination.
[b] The symbols x, x x, and x x x indicate the relative severity of the condition found.

The duration of life of the guinea pigs fed on the cold-pack pear varied from 22 to 50 days and averaged 35.7 days. The animals that lived longer were, as a rule, those that ate the larger amounts of pear. As only a comparatively few cases are under consideration, they show wide variation; averages are used, therefore, to show the general trend of the results.
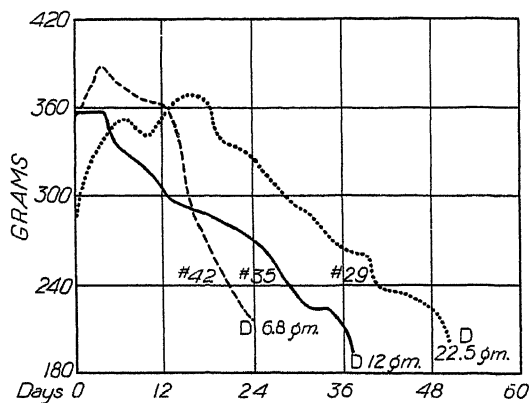
FIG. 3.—Growth curves of three guinea pigs, one eating 6.8 gm., another 15 gm., and the third 22.5 gm., of cold-pack pear daily. The curves are marked "D," indicating that the animals failed to survive the full experiment period of 90 days

DISCUSSION

It has been indicated in this experiment that the minimum protective dose of raw pear is a little more than 10 gm. and less than 15 gm. It was not possible to find amounts of canned pear sufficient to give protection from scurvy. The vitamin C content of open-kettle pear must be practically all destroyed, as the average survival period was no greater than the expected survival period of animals on the basal diet alone, while the antiscorbutic value of cold-pack pear is not so completely destroyed, since the larger amounts usually increased the survival period slightly. Factors entering into the destruction of vitamin C in the home processes of canning pear are probably heat and oxidation.

The P_H of the canned pear was 4, as determined by the colorimetric method, the bromphenol blue, 0.02 per cent aqueous solution being used according to Medalia (*10*).   Harvey (*4*), in his determination with the standard hydrogen electrode equipment, found the P_H of pears purchased in the open market to be 4.2.   LaMer, Campbell, and Sherman (*9*) found the P_H of tomatoes to be 4.3. According to their results less destruction of the vitamin C content in the canning of pears might have been anticipated.

The antiscorbutic value of raw pear might be considered about 25 per cent of the potency of orange juice or of tomato juice, since the minimum protective dose of pear is about 12 gms. as compared to 3 c. c. of tomato juice which, according to Sherman (*11*), is necessary to give complete protection.

The relative richness in vitamin C of certain fruits as given by Sherman (*11*) is as follows:

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Orange juice_____   100
Lemon juice_____   100
Pineapple, fresh, raw_____    70
Lime juice_____    25
Banana_____ 20–40
Apples, raw_____ 10–20
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If orange juice is taken as 100, raw pear would have a potency of 25.

SUMMARY AND CONCLUSIONS

Observations made in this study lead to the following conclusions:

The minimum protective dose of raw pear for guinea pigs is between 10 and 15 gms.   The animals on 10 gms. of raw pear daily gave no indications of scurvy during life, and the autopsies revealed no sign of a vitamin C deficiency.   The only indication was a negligible loss in body weight.   The 15-gm. amounts fed daily gave good protection to the animals and some gain in body weight.

The potency of raw pear is estimated as 25 if orange juice is regarded as 100.

The antiscorbutic factor in pear is destroyed in the open-kettle method of home canning.   This was shown by the fact that the animals fed open-kettle pear lived on the average no longer than the expected survival period when the basal diet alone was given.

The antiscorbutic factor in pear is not completely destroyed by the cold-pack method of home canning.   In no case, however, did the animals fed cold-pack pear survive the experimental period of 90 days.

The storage of fresh pear seemed to make no difference in the antiscorbutic content, since the animals fed fresh pear made the same rate of gain during the later part of the experimental period as they made earlier in the year when they were fed fresh pear that had not been stored.

The California pear was no more potent than the home-grown fruit, as was evidenced by the fact that no marked change in the weight curves of the animals occurred when California pear was substituted for home-grown pear.

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STERILITY AND FERTILITY IN THE STRAWBERRY [1]

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SEX TYPES AND STERILITY

In the cultivated strawberry two sex forms, pistillate and hermaphrodite, are recognized. Although plants of pistillate-flowered varieties are reported to produce stamens occasionally, they have never been observed by the writer to do so. Instead, they produce staminodia, which do not contain even abortive pollen. Staminodia are seen as small white projections surrounding the pistils.

Plants of pistillate varieties commonly set all their flowers. Although plants of hermaphrodite varieties ordinarily produce flowers having both stamens and pistils, some of the flowers of many varieties so not contain stamens. In rare cases also flowers of this form have been produced which lack pistils entirely. (Fig. 1.) Plants of hermaphrodite varieties do not ordinarily set all their flowers; many of the later ones on an inflorescence may be sterile.

There is a marked difference between the fertility of hermaphodite varieties grown to-day and that of those raised 75 years ago (*1*).[2] Varieties grown in 1850 set only 15 to 20 per cent of their flowers; present varieties set on an average about 66 per cent and some set as high as 100 per cent.

Self-sterility and cross-sterility, common in other fruits of the rose family, have not been found in the cultivated strawberry. Pistil sterility of hermaphrodite varieties is the only important type of sterility in this fruit. It is and always has been one of the outstanding problems in the development of the strawberry. (Fig. 2.)

Valleau (*7*) has shown that pollen abortion is common in cultivated varieties of strawberries. Ordinarily this is of little importance commercially among cultivated varieties. In the Glen Mary and Progressive varities, however, lack of pollination, resulting from the scarcity of the good pollen grains, may cause some "nubbins." In all cases thus far observed pollen grains similar in appearance to those of a good species have been found to fecundate fertile pistils of any variety in the same chromosome group.

RELATION OF SEX TYPES TO SPECIES

Longley (*3*) has recently pointed out that the 7-chromosome species, *Fragaria vesca*, *F. nilgerrensis*, and allied forms are characterized by the production of only hermaphrodite flowers, all of which

set. The 21-chromosome species, *F. elatior*, and the 28-chromosome species, *F. chiloensis*, *F. virginiana*, and allied forms have a variety of sex-flower types. Some seedlings in each 28-chromosome species studied bear pistillate and others hermaphrodite flowers, the hermaphrodite-flowered forms exhibiting a series of sex types ranging from those setting all flowers to those setting none.

The cultivated varieties of Europe and America are supposed to have descended in part at least from plants of *Fragaria chiloensis* taken to Europe in 1714 by Frezier. Wilson Popenoe has recently
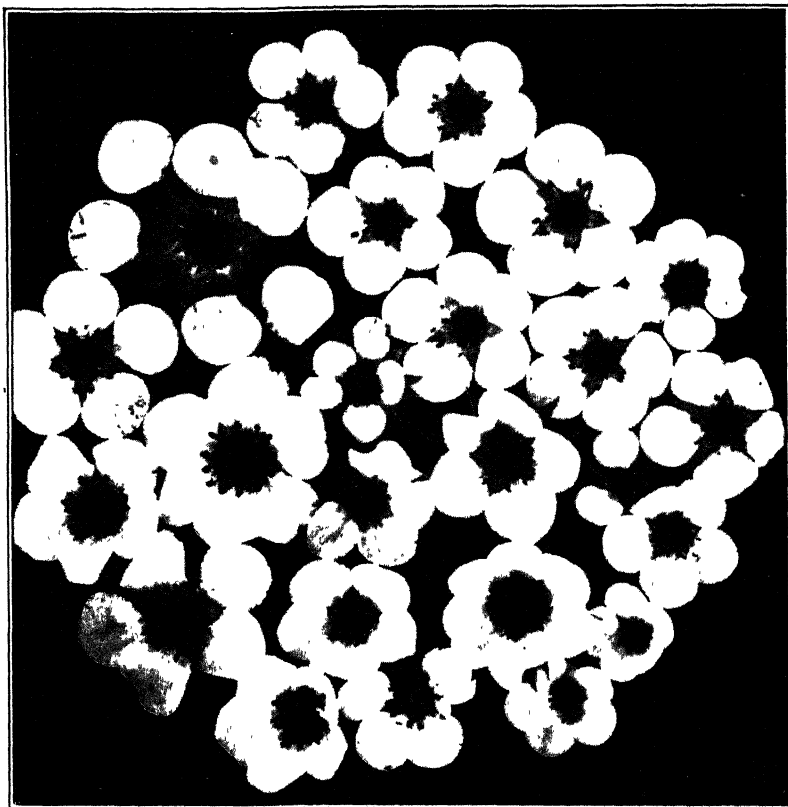
FIG. 1.—Flower types of the Howard 17 strawberry. Flowers with all parts present, some lacking part or all of the stamens, and one lacking pistils, are shown

reintroduced what he regards as the same variety. It is hermaphrodite-flowered and under conditions at Glenn Dale, Md., its flowers are mostly sterile. (Fig. 3.) Of several hundred crosses of *F. chiloensis* with cultivated varieties at Glenn Dale, most showed similar sterility. Though Duchense and many later writers refer to Frezier's plant as pistillate-flowered, Popenoe has stated that he has never seen a pistillate variety of this species under cultivation in any part of South America. All the hermaphrodite seedlings of *F. chiloensis* from the Pacific Coast recently placed under cultivation at the Bell Horticultural Field Station at Glenn Dale, Md. were nearly or

FIG. 2.—Seedlings of *Fragaria virginiana* No. 27 (pistillate) × Marshall that to the left (pistillate) has set all flowers, that to the right (hermaphrodite) has set none. Photograph taken June 25, 1924

FIG. 3.—The Chilean strawberry brought to this country by Wilson Popenoe. It has long been grown in South America and is supposed to be the same variety as that taken to France by Frezier in 1711. Note that four fruits have set seeds and two others have set a few seeds. Thirteen flowers were sterile

entirely sterile. Pistillate seedlings from the same source set all
their flowers. Examination of large numbers of *F. chiloensis* near the
mouth of the Columbia River on both the Oregon and Washington
coasts in 1926 showed the same condition. The pistillate plants set
all their flowers. Most of the hermaphrodite ones were sterile
although some bore more or less fruit.
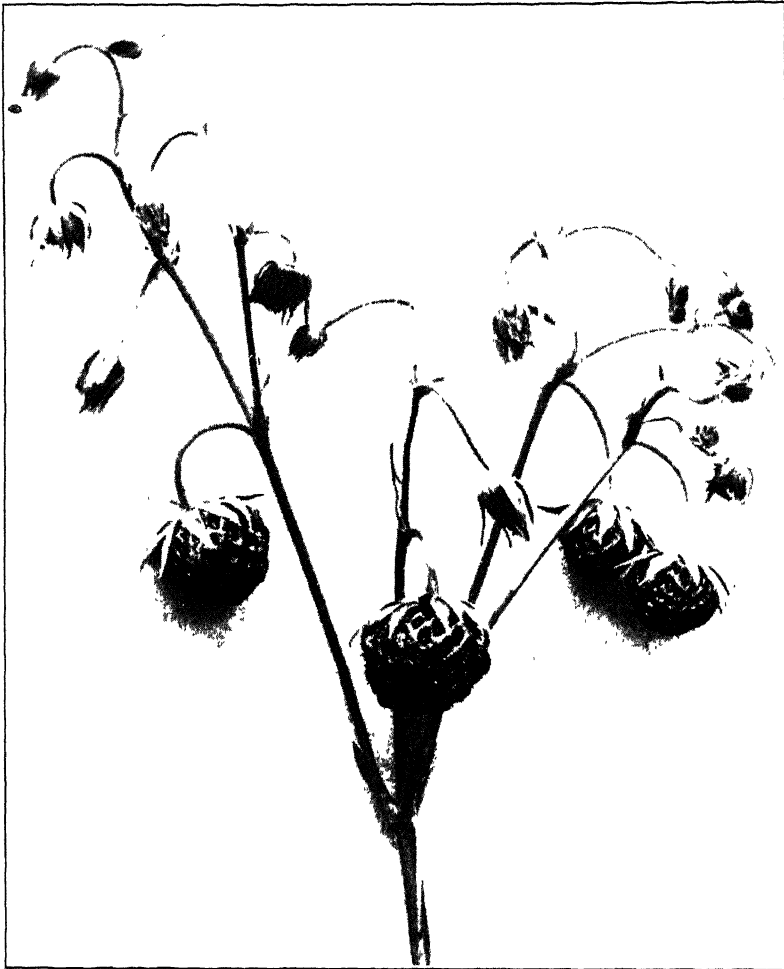
Fɪɢ. 4.—Fruit cluster of Little Scarlet, a variety of strawberry long grown in Europe for jam. It is
probably an American selection of the wild *Fragaria virginiana*. Five flowers out of nineteen set
fruit. On heavy soils at Geneva, N. Y , a much larger percentage sets fruit. Photograph taken
June 12, 1924

To obtain the European strawberry of to-day, selections of *Frag-
aria virginiana* were crossed by the early breeders with *F. chiloensis*.
It has been possible to procure from England the Little Scarlet vari-
ety, one of these early selections. At Glenn Dale, Md., this variety
shows sterility comparable to the Chilean variety introduced by
Popenoe. (Fig. 4.) Crosses of it with cultivated varieties are

apparently even more sterile than similar crosses with the Chilean. Valleau (7) reports that in the wild, a large part of the hermaphrodite forms of *F. virginiana* are sterile while pistillate varieties set all their flowers. *F. chiloensis* and *F. virginiana*, from which cultivated varieties are chiefly or wholly derived, apparently exhibit similar sex types and sterility.

CORRELATION OF STERILITY AND STAMEN PRODUCTION

Valleau (7) points out that pistillate flowers occur on hermaphrodite varieties and that when they occur on hermaphrodite-flowered seed-lings which usually are entirely sterile, such pistillate flowers set fruit. Records previously published (1) show that the pistillate flowers found on hermaphrodite varieties are commonly the first flowers to open on an inflorescence; that is, they are the primary and secondary flowers. The first flowers to open, the primary and secondary ones, are also most likely to set on hermaphrodite varieties. All or commonly all pistillate flowers set fruit, and sterility tends to appear only when stamens are produced.

PISTILLATE FLOWERS ON HERMAPHRODITE VARIETIES

The frequency with which pistillate flowers are found on hermaphrodite varieties has been shown by the writer. Occasionally when cool weather prevails and flowering is delayed these first flowers are not pollinated and the extent of the occurrence of such pistillate flowers assumes some importance.

The occurrence of pistillate flowers on hermaphrodite varieties varies in different years. In 1925, for example, 28 varieties at Glenn Dale, Md., showed pistillate flowers on which none were noted in 1926. Fruit-bud studies (3) indicate that in the primary flowers at least, stamens develop in the fall nearly to the pollen-mother-cell stage, in all but the latest formed plants in northern States. Fall conditions affect considerably the development of the sex organs in certain varieties, inhibiting the formation of anthers in many of the first flowers to differentiate.

INTERMEDIATES BETWEEN HERMAPHRODITE AND PISTILLATE VARIETIES

Valleau (7) has reported intergrades in hermaphrodite varieties from those setting all or nearly all of their flowers to those setting no flowers. These intergrades are in reality males having pistils which are not functional. Heretofore, no instances have been noted in which intergrades between hermaphrodite and pistillate varieties have occurred. Two such intergrades have apparently been found among the selections resulting from the practical breeding work of the Bureau of Plant Industry at Glenn Dale, Md. The first is No. 503, a selection of Ettersburg 445 × Howard 17. A count on this selection, May 11, 1926, early in the blossoming season, showed 38 pistillate flowers, 54 with all stamens abortive, and 74 with one or more good stamens. On May 15 the same plants showed 23 pistillate flowers, 20 with abortive stamens, and 84 with one or more good stamens.

The other selection, No. 799, Portia × Kalicene, had on May 11 only 4 pistillate flowers, 69 with abortive stamens, and 39 with one or

more good stamens. Of these, 29 primary flowers had abortive sta-
mens and 6 had some good stamens. Three secondary flowers were
pistillate, 32 had all abortive stamens, and 24 some good stamens.
One tertiary flower was pistillate, 8 had abortive stamens, and 9 some

FIG. 5—Selection No. 799, Portia × Kaheene, which is apparently an intermediate between a
pistillate and a hermaphrodite variety. Some flowers are pistillate, others have abortive stems
and still others have good stamens. Photograph taken May 14, 1926

good stamens. Although no counts were made, it was observed that
most of the last flowers to open had good stamens It is possible that
these two selections, Nos. 503 and 799, represent intermediate stages
between pistillate and hermaphrodite varieties. (Fig. 5.)

Attention should also be called to a hermaphrodite variety, Rockhill, which, under conditions at Glenn Dale, Md., sets nearly all of its flowers. A count of 20 plants showed an average of 37.2 fruits set, 2.9 flowers not set, and 6.4 flowers, buds, or missing. The set of fruit obtained in the Rockhill compares favorably with that of pistillate varieties. Near by, Sample, a pistillate variety, set an average of 8.6 fruits, 2.3 did not set, and 0.3 were flowers, buds, or missing. Selections of crosses of Rockhill with other varieties show a remarkably high percentage of flowers that set. They also show many pistillate flowers and flowers containing abortive stamens. Whether such a variety should be listed as an intermediate between a pistillate and a hermaphrodite variety is not yet clear.

CHANGE OF SEX

One variety, Ettersburg 121, has, on some soils in Oregon, been reported as the most productive sort, while on other soils a large percentage of its flowers have been sterile and it has been so unproductive as to be unprofitable. After a careful survey, Schuster (6), of the Oregon Experiment Station, found it to be most productive on rather heavy soils and least productive on sandy soils. When grown on sandy soil at Glenn Dale, Md., rarely does 1 flower in 50 of the Ettersburg 121 set fruit. The writer's attention has been called by W. A. Taylor, of the Bureau of Plant Industry, to a similar difference in productiveness in the Cumberland Triumph grown some 30 years ago, which was productive on the heavier black soils in the eastern part of the United States but was unproductive on light soils.

C. C. Georgeson, Director of the Alaska Agricultural Experiment Station, has reported (2) a change in fertility in the beach strawberry, *Fragaria chiloensis*, which was unproductive when set in good garden soils but when transplanted to sterile volcanic soils became productive. Little Scarlet sets few flowers on sandy soils at Glenn Dale, Md., but on clay soils at Geneva N. Y., it sets most of its flowers. These observations indicate that sterility in the strawberry may be greatly influenced by the soil type. Just what influence the soil does have, however, is difficult to determine.

Valleau (7) also cited instances of changes in the sex type of varieties. For example, plants of the Glenville variety which set fruit only occasionally when grown in the greenhouse set over 50 per cent of its flowers the following summer.

European varieties, when grown at Glenn Dale, Md., exhibit sterility to a marked degree. Few of the scores of varieties introduced in the past few years set more than 1 to 3 flowers to each inflorescence. (Fig. 6.) In this case the length of day rather than soil type is the most marked environmental difference, the days being much longer in Europe during most of the growing period than in the United States. Whether difference in length of day or some other influence at the time of fruit-bud formation is actually the cause of the difference in sterility is of course only speculative.

Records have previously been published showing the extent of sterility in cultivated varieties (1). Additional records are given in the accompanying tables, as follows: Table 1 shows the average number of fruits and of flowers not setting on 20 hermaphrodite varieties for which records were obtained in 1925 and 1926; Table 2 gives the same data for other varieties.

TABLE 1.—*Average number of fruits and of flowers not setting per plant of 20 varieties of strawberries in 1925 and 1926, Glenn Dale, Md. The data for 1925 are averages of 10 plants, and for 1926 of 20 plants, except as noted*

Variety	1925		1926		Remarks
	Fruits	Flowers not set	Fruits	Flowers not set	
Americus	7.4	7 2	7.2	8.7	
Big Wonder	6 6	23.4	4.3	18.8	Average 20 plants, 1925.
Brandywine	17 1	4 4	10 2	13 0	
Campbell	23.1	9.3	17 7	12.6	
Clark	6 0	9 2	2.3	3.5	
Ekey	9.2	18 2	5.0	13.3	
Excelsior	10 9	1.8	5 7	2.3	Average 25 plants, 1925.
Freemont Williams	5.6	4 5	6.6	4.9	
Glen Mary	11.1	4.1	11 6	4.3	
Gold Mine	15.2	1.0	9.4	2.7	
Howard 17	16.4	2.7	6.7	1.8	Average 36 plants, 1925; 305 plants, 1926.
Judith	13.8	6 2	6.2	3.0	
Lady Corneille	12.6	9.7	6.1	4.4	
Late Stevens	6.1	2.5	4.4	4.8	
Marvel	16.1	7.0	11.2	3.8	
Mascot	10 7	8 4	6 0	3.0	
McAlpine	13.9	11 0	4.7	4 8	
Ozark	10 3	1.0	12.8	1.0	
Pearl	13 0	1.2	19.4	.5	
Progressive (Champion)	16 0	1.8	14.7	1 1	
Average	12 6	6.7	8 6	5.6	
Percentage of flowers not set		36		39	

TABLE 2.—*Average number of fruits and of flowers not setting per plant of strawberry varieties in 1925 and 1926, Glenn Dale, Md. The data for 1925 are averages of 10 plants, and for 1926 of 30 plants, except as noted*

Variety	1925			1926			Remarks
	Fruits	Flowers not set	Others	Fruits	Flowers not set	Others	
Abington	19.3	8.0	0.9				
Ananas de Guemene (P.)				9.3	0	1.6	
Aroma				3.7	3.1	— 8	Average 100 plants.
Plants rooting by July 15, 1925.				6.0	3.9	—.1	Average 19 plants.
Plants rooting July 15–Aug. 1, 1925.				4.5	3.4	.2	Average 19 plants.
Plants rooting Aug. 1–Sept. 9, 1925.				2.7	2 8	.0	Average 62 plants.
Benson	10.5	11.0	1.3				
Big Late (P)	11 2	.9	3.6				
Bun	22.7	7.0	6.1				
Burrill				5.4	8.4	—1.9	
Chesapeake	12 4	.3	2.4				Average 146 plants
Cooper	13.1	3 9	1.8				
Delicious	13.6	10.7	2.5				
Doctor Hogg	15.4	1.6	1.5				
Dunlap (potted)				10.7	9.3		Average 69 plants.
Dunlap (in field)				4 7	6.9	7	Average 307 plants.
Plants rooting by July 15, 1925				10 1	9 2	2.1	Average 46 plants.
Plants rooting July 15–Aug. 1, 1925.				6 1	8 7	1.0	Average 84 plants.
Plants rooting Aug. 1–Sept. 9, 1925.				2.5	5 3	.2	Average 177 plants.
Eaton				8 2	1.8	.7	
Ettersburg No. 80	11.6	10.7	2.5				
Ettersburg No. 121	.2	17.3	10.7				
Ettersburg No. 121				1.9	5 0	.3	Average 61 trusses.
Ettersburg No. 445 (P)	10 2	0	2.9				
Fendall (P)	20.3	.1	3.0				
Gardners No. 999	9.0	14.7	1.1				
Haverland (P)	33.9	2.9	2.3				
Heflin	13.6	13.8	2.2				

TABLE 2.—*Average number of fruits and of flowers not setting per plant of strawberry varieties in 1925 and 1926, Glenn Dale, Md. The data for 1925 are averages of 10 plants, and for 1926 of 30 plants, except as noted*—Continued

Variety	1925			1926			Remarks
	Fruits	Flowers not set	Others	Fruits	Flowers not set	Others	
Howard 17				6. 7	1. 0	3. 0	Average 305 plants.
Plants rooting by July 15, 1925				9. 6	6	2. 9	Average 59 plants.
Plants rooting July 15-Aug 1, 1925.				9. 5	8	2. 3	Average 76 plants.
Plants rooting Aug. 1-Sept 9, 1925.				4 5	1 3	1. 6	Average 170 plants.
Howard 25 (P)	45. 2	2 9	2. 2				
Kahcene				8 2	1 1	. 4	Average 31 trusses.
Kelloggs Prize (P)	19 9	. 5	2. 7	12 4	. 9	. 5	
Klondike				3. 8	. 7	8	Average 303 plants.
Plants rooting by July 15, 1925.				5. 2	. 3	. 8	Average 80 plants.
Plants rooting July 15-Aug. 1, 1925.				4. 7	. 7	. 9	Average 85 plants.
Plants rooting Aug. 1-Sept 9, 1925				2. 3	. 9	7	Average 138 plants.
La Constante	3 0	39. 8	1 9				
La Perle	9. 1	8	2. 6				
Lupton	5 9	0	. 1				
Michel	10 4	3. 2	2 1				Average 25 plants.
Missionary				4. 2	2. 6	. 6	Average 488 plants.
Plants rooting by July 15, 1925.				5 2	2 8	1 1	Average 143 plants.
Plants rooting July 15-Aug. 1, 1925.				4 5	2. 8	. 7	Average 142 plants.
Plants rooting Aug. 1-Sept. 9, 1925.				3. 3	2. 3	. 2	Average 203 plants.
New York	6 8	8 7	1 8				
Orem	7. 1	7. 1	1 2				
Portia (P)				8 6	0	. 3	Average 100 plants.
Rockhill				37 2	2. 9	6 4	Average 20 plants.
Sample				8. 6	2. 3	. 3	Average 100 plants.
Plants rooting by July 15, 1925.				13. 3	2. 3	. 7	Average 23 plants
Plants rooting July 15-Aug. 1, 1925.				9. 1	2. 1	. 2	Average 27 plants.
Plants rooting Aug. 1-Sept. 9, 1925.				6. 2	2. 5	. 2	Average 50 plants.
Shropshire	8 6	1 4	1 2				
Success	15. 5	1 9	2. 4				
Tennessee				8. 4	4 3	2. 6	Average 29 plants
The Queen	4. 4	3. 5	1. 5				
Van Fleet No. 125				6 2	3. 1	1. 0	Average 100 plants.
Van Fleet No. 125 (with stable manure mulch).				8 8	3. 2	1. 5	Average 100 plants.
Vanguard				5. 8	2. 1	. 5	
Vineland No. 19322				6 0	. 7	. 4	
Warfield (P)	31. 2	4. 3	2. 6	13. 1	2. 9	. 3	
White Pineapple	1 2	18. 4	2				
Average 7 pistillate varieties.	31 7	1. 7	2. 8				
Average 21 hermaphrodite varieties.	10. 2	8. 8	2 2				

The average number of flowers not setting in 20 varieties of strawberries, as shown in Table 1, was 6.7, or 36 per cent in 1925, as compared to 5.6, or 39 per cent, in 1926—hardly a significant difference. For all varieties the correspondence for the two years is remarkable. Attention is called, however, to certain exceptions. Thus the Brandywine showed a marked change, producing 17.1 fruits and 4.4 flowers not set in 1925 and 10.2 fruits and 13.0 flowers not set in 1926. Late Stevens also showed a marked difference. In 1925 the number of flowers not setting in this variety was less than one-half the number of fruits not setting, as contrasted with more

flowers than fruits not setting in 1926. Both Brandywine and Late Stevens showed a decided increase in sterility in 1926 over 1925.

The close agreement in both years in such varieties as Big Wonder, which showed a relatively large number of flowers not setting; in Americus with almost an equal number of flowers not setting and of fruits; and in Gold Mine, Howard 17, Ozark, and Pearl, which showed few flowers not setting, is noteworthy.

FIG. 6.—A fruit stem of Utility, an English strawberry, showing the primary and two secondary berries set; the rest did not set

At the bottom of Table 2 is given the average of the 7 pistillate and 21 hermaphrodite varieties on which records were obtained in 1925. Pistillate varieties produced an average of 1.7 flowers and hermaphrodite varieties 8.8 flowers, which did not set. The difference between the number of pistillate and hermaphrodite varieties not setting flowers is 6.1, or 37 per cent. It is probable that this difference represents the actual number of sterile flowers which would not set under any conditions.

EFFECT OF FALL CONDITIONS ON STERILITY

In the northern and northeastern part of the United States, the fruit buds of the strawberry differentiate in the autumn. The first traces of fruit buds were found by Ruef and Richey (5) in Iowa by the middle of September. Fruit buds develop rapidly and show well-developed pistils and stamens before winter. In some varieties, anther development in the primary and secondary flowers proceeds nearly to the pollen-mother-cell stage before winter.

Ventilated boxes constructed to exclude all light have been placed over dormant plants in the field at Glenn Dale, Md., and kept over them until the blossoming season. The strawberries came into flower in the dark, but in no case was there any apparent development of stamens or petals. Sepals developed to a limited extent

Fig. 7.—The Howard 17 variety, brought into flower in late winter, showing 19 flowers lacking good stamens, 6 of which also lack developed petals. Even the sepals are much smaller than usual. Photograph taken April 11, 1925

only. Other dormant strawberry plants grown in dark constant-temperature chambers behaved in the same way. Light seems to be necessary for the development at least of stamens and petals. Figure 7 shows a plant brought into flower in the short days of winter. Many of the flowers did not produce any good stamens and some did not produce any petals. Usually, under such conditions, the pistils were functional and set fruit although in some cases this did not seem to be the case. It would appear that pistil development proceeded relatively farther than stamen development in the fall.

Gardner in Missouri found no change in the number of fruits on plants of the Dunlap variety following the application of nutrients in the spring. In Michigan, Loree (4) increased the set of fruit on plants of the Dunlap variety grown in a very light sand by the application of nitrogen in the spring of the fruiting year. Unfertilized plants set 22.7 per cent of their blossoms, while plants fertilized with

nitrate of soda just before blossoming set 40 per cent. Set of fruit, however, may not be an index of pistil sterility in his experiments. Plants receiving spring applications of nitrogen make a vigorous growth and thus supply ample water and food to their flowers, which weak plants are less able to do. Even though the pistils of the flowers of the weaker plants in Loree's experiment may have been fertile they may not have been supplied with the essentials for further development. A similar test of a pistillate variety might perhaps give similar results even though all the pistils of the variety were fertile. At Glenn Dale, Md., no spring treatment given seems to have affected the proportion of sterile flowers in the spring.

Frost often kills the pistils of the first flowers on inflorescences of varieties which commonly set only these first flowers. When this occurs no later flowers appear to set. The pistils of the first flowers may be killed when the buds are very small, but in no case has any change in the setting been observed. In a seedling (*Fragaria platypetala* × Klondike), which commonly set only the first flowers on an inflorescence, all the primary flowers were picked off before they opened. No increase in the set of later flowers was obtained. Under most conditions pistil sterility seems to be determined in the fall.

A hermaphrodite selection of supposedly pure *Fragaria virginiana* collected by D. N. Shoemaker in the mountains of North Carolina is shown in Figure 8. Its basal truss, which had 6 flowers not set and 19 fruits set, is shown in Figure 9. Figure 10 shows the three higher trusses which produced 20 fruits and also 13 flowers which did not set. As the inflorescence of the strawberry is terminal, new growing points must form before other fruit buds can develop. The basal truss shown is the center one and developed first; new growing points were then laid down, which later formed fruit buds; but on these later-formed inflorescences a slightly larger percentage of the flowers were sterile. Figure 11 shows a potted plant of the Dunlap with a basal and high-branching truss, the first having 5 fruits and 2 sterile flowers and the latter 5 fruits and 5 sterile flowers. Records taken on 31 potted plants of this variety having two or more trusses gave 46 per cent sterile on the earlier trusses and 62 per cent sterile on the later ones.

Examination of other cultivated varieties reveals the same condition as in the Dunlap; the later-formed trusses show greater sterility than those formed earlier. Figure 12 shows a crown of the Howard 17 variety with three trusses, the center one of which set 5 fruits one of which was frosted. The other trusses produced 5 fruits and 4 sterile flowers. It is evident that the later-formed fruit buds exhibit a greater percentage of sterility than do those formed earlier. Whether this is due to climatic, nutritional, or other conditions at the time of fruit-bud formation is not clear.

Ruef and Richey (5) reported that the earlier-rooting runner plants formed fruit buds first and that these buds developed to a greater degree in the fall than did those formed later. Table 2 summarizes a record of the number of fruits and sterile flowers produced in 1926 on strawberry plants of leading standard varieties, the runners of which were rooted on different dates in 1925. Thus, on May 24,

FIG. 8.—One plant of selection No. 13 of *Fragaria virginiana*, which has produced several fruit clusters. The earlier flowers set; many of the later ones did not
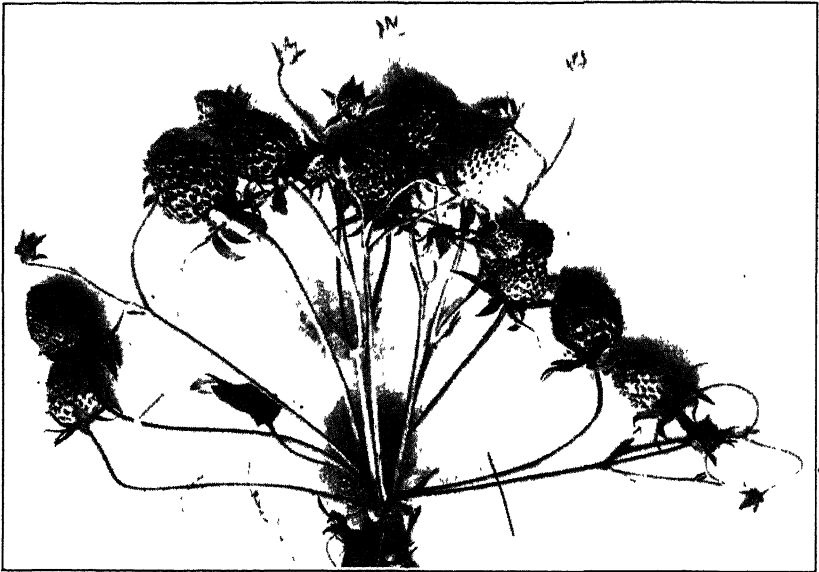
FIG. 9.—The basal truss of the plant shown in Figure 8. Nineteen fruits set and 6 flowers did not. Photograph taken June 12, 1924

FIG. 10.—Three later-formed trusses of the plant shown in Figure 8. To the right are shown 8 fruits and 5 flowers not set; in the center 6 fruits, 1 flower, and 3 flowers not set; to the left 6 fruits and 5 flowers not set; a total of 20 fruits, 1 flower, and 13 flowers not set

FIG. 11.—Dunlap plant with basal and high-branching clusters.   The basal cluster has 5 fruits and 2 sterile flowers; the high cluster 5 fruits and 5 sterile flowers.   The basal cluster has also earlier-maturing fruit

1926, counts were made on 303 plants of the Klondike, the most important commercial variety in America, which were rooted on different dates the previous year. Eighty plants, rooted on or before July 15, produced 418 fruits, 21 flowers not set, and 65 others (average 5.2, 0.3, 0.8). Eighty-five plants, rooted between July 15 and Au-

FIG. 12.—Howard 17 strawberry plant with 3 fruit clusters. The center was the terminal and formed 5 flowers, one of which was frosted. Later a new growing point developed on each side and a terminal inflorescence formed on each point On that to the left 2 fruits and 3 sterile flowers, and on that to the right 3 fruits and 1 sterile flower developed

gust 1, produced 400 fruits, 59 flowers not set, and 80 others (average 4.7, 0.7, 0.9); 138 plants rooted August 1 to September 9 produced 322 fruits, 129 flowers not set, and 102 others (average 2.3, 0.9, 0.7).

Of the five hermaphrodite sorts, Klondike, Howard 17, Aroma, Dunlap, and Missionary, the average was 60 per cent fruits and 28 per cent flowers not set for the plants rooted earliest; 58 per cent fruits and 32 per cent flowers not set for late July plants; and 51 per

cent fruits and 41 per cent flowers not set for plants rooted in August and early September. In every variety an increase in the percentage of flowers not setting in the later-rooted plants is evident, although the number and percentage of flowers not setting differs with varieties. (Figs. 13 and 14.)

This increase in unfruitful flowers may be attributed to the time of formation of fruit buds, the later-rooting plants forming fruit buds last, or to the relative vigor and food supply of the plants on which
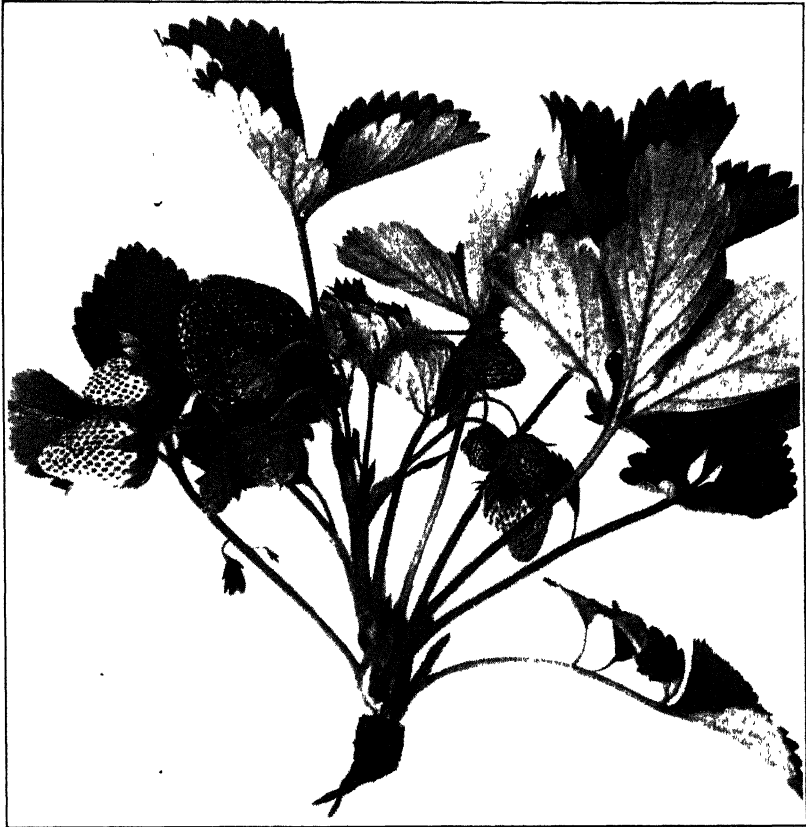
FIG. 13.—Plant of Howard 17 strawberry, rooted in July, which produced 12 fruits and 1 sterile flower

they were borne. If time of fruit-bud formation is the important factor then it may be assumed that the influence of one or more climatic conditions effective at the time of fruit-bud formation directly or indirectly determines the extent of sterility.

From a comparison of the extent of sterility during different years it is evident that factors such as sunlight, temperature, evaporating power of the air, water-supplying power of the soil, length of growing season, or other similar factors were sufficiently different during the fall periods to bring about a change in the degree of sterility in some varieties.

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SUMMARY

The two species, *Fragaria chiloensis* and *F. virginiana*, from which cultivated strawberries have largely or entirely developed, have the same chromosome number, and exhibit the same pistillate and hermaphrodite flowered sex types; the latter type shows great diversity from males possessing no functional pistils to those setting many of their flowers.

Under the conditions which prevail at Glenn Dale, Md., plants of the particular varieties of *Fragaria chiloensis* and of *F. virginiana*

Fig. 14.—Howard 17 strawberry plant, rooted in September, 1925, which produced 8 fruits and 3 sterile flowers

from which modern varieties may have been chiefly derived are largely sterile.

Two selections are reported as intermediates between hermaphrodite and pistillate forms, the first representatives of such a group yet reported. A hermaphrodite variety, the Rockhill, is reported as behaving, in the setting of its flowers, more like a pistillate than a perfect-flowered variety.

Evidence that sex expression in hermaphrodite varieties is changed by soil conditions has been presented by others and apparently is confirmed. Records covering the setting of flowers and fruit of 20 varieties for both 1925 and 1926 and of many other varieties for one. of these years are presented. They show a remarkable correspondence in percentage of sterility in varieties for the two years.

When the floral parts abort or do not develop because of unfavorable conditions the stamens are the first to be affected. Under more extreme conditions petals are lost, sepals next, while pistils are the least likely to be affected.

Although it may affect the actual set of fruit, spring treatment appears not to affect the number of sterile flowers.

The earlier formed trusses and the earlier rooted plants of the previous season appear to have less sterility than those formed or rooted later.

The character of the fall season is considered one of the important causes of fertility and sterility in this fruit.

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A STUDY OF THE FACTORS DETERMINING QUALITY IN SWEET CORN [1]

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INTRODUCTION

The importance of quality in sweet corn as affecting its successful manufacture and sale as a canned product is coming to be more fully appreciated. A thorough understanding, therefore, of just what constitutes quality and what those factors are by which it is affected is desirable.

It is known that seasonal and climatic factors, through their influence on the rate at which the corn develops and matures, play an important part in determining the quality of the canned product.[2] It is apparent also that certain characteristics possessed by sweet corn as distinct from field corn are likewise of very great importance.

Among the various types of corn used as human food a wide variation is known to exist. Earlier investigations [3] on the relation of the variety of corn to the quality of the canned product brought to light fundamental differences in the constitution of sweet and field corns which were reflected directly in the physical appearance and table quality of their canned products. In order, therefore, to gain a better understanding of what constitutes quality, and of the relation of varietal differences to it, the field of the investigations here discussed was extended to include not only the sweet but also representatives of the flint, dent, and flour corns, waxy maize, and other well-known or special types not previously investigated. The results of this study are set forth in the present paper.

To indicate his conception of the probable relationship of the different types of corn, Sturtevant [4] in his taxonomic grouping of the different varieties of corn placed the podded varieties first as the most primitive. Pop, flint, dent, and soft or flour corns in the order named were included in his grouping. He was uncertain as to the relationship of the sweet varieties to the other types. Waxy maize was little known until comparatively recently and hence was not considered in Sturtevant's work.

Whether or not one is justified in assuming any evolutionary relationship between the different types of corn, there are certain physical characteristics that suggest such a relationship. This fact should be kept in mind in the discussion of the present findings.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 5
Mar. 1, 1927
Key No. G-562

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In the flint corns the endosperm is characterized by a starchy core surrounded by a hard, flinty portion that varies somewhat in thickness in the different varieties. In the dent corns the sides of the kernel are composed of a cornaceous portion, the starchy core being central in location and extending to the tip of the kernel. It is to the shrinkage of this starchy portion that the denting of the kernel is due. In the soft or flour corns little of the tough horny element is present, the contents of the endosperm being made up almost entirely of soft, starchy tissue.

Sweet corn is characterized by a translucent, hard, wrinkled or shriveled endosperm, the shriveling being due apparently to the nature of the polysaccharides contained in it and the manner in which they are laid down. In physical appearance the waxy maize resembles more nearly the corn of the flint varieties, having a hard full kernel. The kernel contents, however, present a waxy appearance when the kernel is split. In chemical composition it more closely resembles the sweet varieties, particularly with respect to the water-soluble portion.

PLAN OF INVESTIGATION

The investigation was carried on during the season of 1925 in the fields and laboratories of the Arlington Experiment Farm, Rosslyn, Va. The location of the test plots and the cultural practices followed in this study were as nearly identical with those of the earlier studies [5,6] as seasonal conditions would permit. The methods of observation, testing, and analysis also were similar.

Nine varieties were studied during the season: Two flour corns, Mandan White and Yellow Assiniboine, from North Dakota; two flint corns, Longfellow and Rhode Island White, from New England; a variety of late sweet corn, which originated in Guatemala; an unnamed variety of waxy maize obtained from the Office of Cereal Crops and Diseases, Bureau of Plant Industry; Second Early Adams, a dent variety widely used by market gardeners for the roasting-ear trade, and obtained from a local seedsman; and two well-known varieties of sweet corn, Golden Bantam and Stowell's Evergreen, from Pennsylvania. The two last-named varieties were planted as checks on the others and also to furnish additional data on these two varieties for comparison with similar data accumulated over a period of several years.

With the exception of three varieties, the plantings in the test covered by this discussion were made May 16. Golden Bantam, Stowell's Evergreen, and Second Early Adams were planted June 26.

To permit of thinning to a uniform stand when the plants were sufficiently well developed, a heavy seeding was made. Standard cultural practices were followed throughout the growing season.

As in previous studies, the rate of development of the corn and the tagging of ears were recorded to facilitate later sampling.

Sampling for chemical analysis, for determination of yields and degree of toughness, as well as for canning tests, was performed as usual.

[5] CULPEPPER, C. W., and MAGOON, C. A. Op. cit.
[6] MAGOON, C. A., and CULPEPPER, C. W. Op. cit.
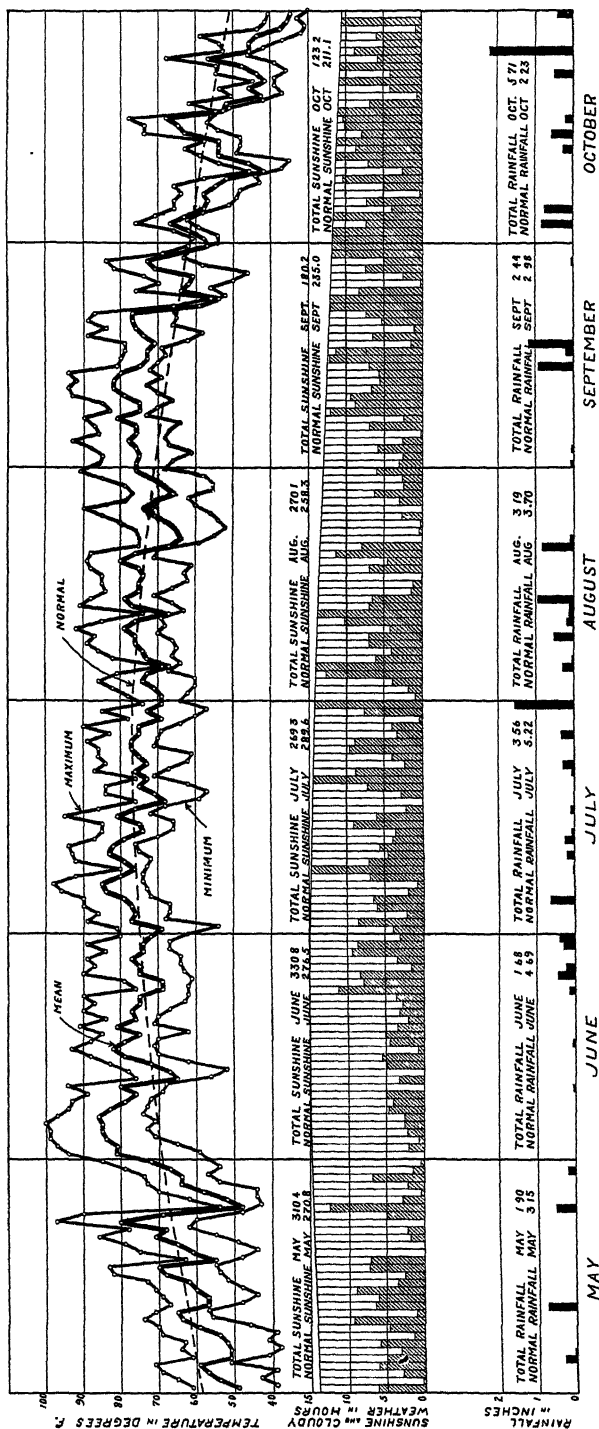
Fig. 1.—Climatic or meteorologic conditions under which corn of different varieties was grown at Arlington Experiment Farm, Rosslyn, Va., 1925

SEASONAL CONDITIONS

The seasonal climatic conditions under which the corn was grown are shown in Figure 1, which gives the meteorological data for the season of 1925 at the Arlington Experiment Farm.

Two outstanding features mark the season—an unusually long period of summer temperature and a considerable deficiency of rain during the growing season.

During the month of May the temperatures showed sharp fluctuations. On May 23, for instance, the summer temperature of 97° F. was recorded. Within 48 hours the maximum temperature dropped to 54°, a variation of 43°. During the latter part of May the temperature rose steadily, and on the 5th of June reached the highest maximum of the summer. The mean daily temperature for June was, with very few exceptions, above normal. During July it remained close to normal. It was slightly below normal during the latter half of the month and the early part of August. During August the temperature showed only slight variation from normal. Summer temperatures and almost unbroken summer conditions prevailed from June 1 to September 21. The latter part of September and the month of October showed the usual sharp fluctuations in temperature.

As already stated, the rainfall was below normal during the spring and early summer. The rainfall during May was only 60 per cent of normal. June showed the most pronounced shortage, the total for the month being 1.68 inches, or about 36 per cent of normal. In July the rainfall was 68 per cent of normal. Approximately one-half of this rain occurred on July 31. The rainfall for August and September was about normal in total amount, although the period from August 23 to September 13 was almost entirely rainless. October had more than twice its normal rainfall, the total for the month amounting to 5.71 inches. The sunshine for the entire season did not vary far from normal.

In the study of sweet-corn behavior it is important that seasonal conditions be taken into consideration since, as has been shown by the writers in an earlier study,[7] temperature has a profound effect upon the rate at which growth and maturity proceed, and influences to some extent also the relative proportions of the different chemical constituents in the kernel.

While rainfall has no apparent effect on the rate at which maturity advances or on the chemical composition of the corn, it does have a direct influence in determining crop yields. Indirectly, through its effect on soil temperatures, rainfall affects the vital processes in the corn kernels.

No specific effect of sunshine on sweet corn has as yet been discovered.

FLOWERING RECORDS

The flour corns, Mandan White and Yellow Assiniboine, were the first to reach the silking period, and the records of these two varieties were entirely similar. Silks were first observed on July 7, 52 days after planting. The silking continued over a period of approximately two weeks, although in both cases the peak of silking occurred on July 8.

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[7] MAGOON C. A., and CULPEPPER, C. W. Op. cit.

The flint varieties, Rhode Island White and Longfellow, were next to flower. Tasseling began on July 13, and on July 17, 62 days after planting, the first silks appeared. The peak of silking in the Longfellow was reached on July 26 and in Rhode Island White on July 28. Silking extended over a period of about three weeks.

The Guatemalan sweet variety began to tassel on July 20, and the first silks appeared on July 30, 75 days after the date of planting. Silking continued for about four weeks, the nearest approach to a peak occuring on or about August 16.

The plot of waxy maize began tasseling on July 21. The first silks were observed on August 9, 85 days from the date of planting. Silking continued for about three weeks, the peak being reached on August 20.

Second Early Adams, Golden Bantam, and Stowell's Evergreen, planted 41 days later than the varieties just mentioned, came into flower very much later than they would have if planted at the same time as the others. Second Early Adams and Golden Bantam each began tasseling on August 5. Their first silks appeared on August 11, 46 days after planting. Silking records were continued for a period of only two weeks. The peak of silking for Golden Bantam was reached on August 19, and for Second Early Adams August 20.

Stowell's Evergreen began tasseling on August 11. Silking started on August 19, 54 days from the date of planting. Records were discontinued after 17 days of silking, the peak being reached on August 31.

While the last three mentioned varieties were planted later and came to flower at a later date than the other varieties, it is doubtful if the actual length of time required for them to come to flower was very different from what it would have been had they been planted at the same time as the others. This assumption is based primarily on the fact that the temperature of the growing periods of both groups of corn as shown in Figure 1 was remarkably uniform.

The ear and cut corn records made in connection with the experiment under discussion are presented in Table 1.

TABLE 1.—*Rate of development of ears of different types and varieties of corn grown at the Arlington Experiment Farm, Rosslyn, Va., 1925*

Type or variety	Date of sampling	Age in days	Number of ears taken	Average weight of ears in grams	Average weight of husked ears in grams	Average weight of cut corn per ear in grams	Per cent of husk	Per cent of cob	Per cent of cut corn	Remarks
Mandan White (flour)	July 24	6	8	82.7	30.0	12.5	63.7	21.1	15.2	
	July 24	10	10	142.2	76.0	14.8	46.5	43.0	10.5	
	July 30	15	3	155.0	100.8	58.3	35.0	27.4	37.6	
	July 29	20	7	189.2	125.7	74.3	33.5	27.1	39.4	
	Aug. 6	24	9	193.3	125.6	81.7	35.0	22.7	42.3	
	Aug. 10	30	8	193.7	140.6	86.2	27.4	28.0	44.6	
Yellow Assiniboine (flour)	July 24	6	7	81.0	25.7	10.0	68.3	19.4	12.3	} Very poor pollination.
	July 24	10	7	109.2	61.7	10.0	43.5	47.3	9.2	
	July 30	15	1	215.0	130.0	70.0	39.5	27.8	32.7	
	July 29	20	7	185.7	109.9	62.1	40.8	25.7	33.5	
	Aug. 6	24	8	177.5	113.7	73.7	35.9	22.5	41.6	
	Aug. 10	30	6	155.8	112.5	58.3	27.7	34.7	37.6	
Longfellow (flint)	July 27	5	13	159.6	55.8	14.5	65.0	25.9	9.1	
	Aug. 3	10	13	262.2	118.0	28.0	55.0	34.3	10.7	
	Aug. 7	15	6	286.6	155.8	41.6	45.9	39.8	14.3	
	Aug. 14	20	5	420.0	232.0	102.0	44.7	30.9	24.4	
	Aug. 24	25	8	326.1	196.8	110.6	39.6	26.4	34.0	
	Aug. 28	30	7	334.2	218.5	140.7	34.6	23.2	42.2	
Rhode Island White (flint)	July 28	5	14	148.5	45.7	16.8	69.2	19.4	11.4	
	Aug. 3	10	11	178.1	136.7	25.4	23.2	62.4	14.4	
	Aug. 12	15	10	261.5	156.0	42.5	40.3	43.4	16.3	
	Aug. 14	20	5	388.0	240.0	112.0	38.1	32.9	29.0	
	Aug. 24	25	8	324.5	219.9	126.2	32.2	28.8	39.0	
	Aug. 28	30	7	378.5	245.7	151.4	35.0	24.9	40.1	
Guatemalan (sweet)	Aug. 13	5	8	165.0	62.5	25.0	62.1	22.7	15.2	} Scanty husks.
	Aug. 13	10	6	190.0	95.9	20.9	49.4	39.9	10.7	
	Aug. 25	15	7	315.0	152.1	70.7	51.7	25.8	22.5	
	Sept. 1	20	7	278.5	217.1	157.8	22.0	21.2	56.8	
	Sept. 9	25	7	308.5	182.8	117.1	40.7	21.2	38.1	
	Sept. 17	30	3	295.0	251.7	193.4	14.6	19.7	65.7	
Second Early Adams (dent)	Aug 19	5	15	166.0	37.4	15.4	66.0	20.0	14.0	
	Aug. 25	10	10	171.0	86.0	26.0	49.7	35.0	15.3	
	Aug. 31	15	6	213.3	121.7	46.7	42.9	35.1	22.0	
	Sept. 9	20	7	300.0	191.4	104.3	36.2	29.0	34.8	
	Sept. 14	25	6	308.3	215.0	135.0	30.2	25.9	43.9	
	Sept. 17	30	6	315.8	226.7	145.9	28.2	25.5	46.3	
Waxy maize	Aug. 24	5	13	149.6	48.4	16.9	67.6	21.0	11.4	
	Aug. 24	10	8	102.5	68.8	21.3	64.2	24.6	11.2	
	Aug. 3	15	8	237.5	90.0	41.9	62.1	20.2	17.7	
	Sept. 9	20	9	276.6	116.6	71.1	57.8	16.4	25.8	
	Sept. 18	25	7	250.0	112.9	81.5	54.8	12.5	32.7	
	Sept. 21	30	6	201.6	128.3	102.5	36.3	12.8	50.9	
Golden Bantam (sweet)	Aug. 25	5	15	73.3	18.0	7.3	75.4	15.0	9.6	
	Aug. 25	10	13	120.7	52.2	26.9	56.7	20.9	22.4	
	Aug. 27	15	8	132.5	72.5	33.8	45.2	29.2	25.6	
	Aug. 31	20	7	148.5	91.4	52.9	38.3	26.1	35.6	
	Sept. 7	25	5	185.0	114.0	79.0	38.3	18.8	42.9	
	Sept. 16	30	5	168.0	113.0	81.0	32.6	19.0	48.4	
Stowell's Evergreen (sweet)	Sept. 5	10	8	236.2	115.0	38.8	51.3	32.2	16.5	
	Sept. 11	15	7	400.0	217.1	107.1	45.7	27.5	26.8	
	Sept. 14	20	7	518.0	358.0	214.0	30.8	27.9	41.3	
	Sept. 18	25	7	415.0	286.5	177.9	30.9	26.1	43.0	
	Sept. 28	31	5	540.0	400.0	284.0	25.9	21.4	52.7	

The results of tests of the corns for toughness at different stages of maturity are of considerable interest and are shown in graphic form in Figure 2.

It will be observed that the different varieties formed two distinct groups with respect to toughness: (1) The flour, flint, and waxy varieties, which became extremely tough as maturing proceeded; and (2) the sweet varieties and Second Early Adams, a dent variety, none of which exceeded the 420-gram mark at 30 days of age.

The grouping of the Second Early Adams with the sweet varieties, in respect to tenderness, is of particular interest. This variety is really a field corn belonging to the dent group. In earliness it corresponds closely with Golden Bantam. This fact, together with its relative tenderness, probably accounts for its wide use in the roasting-ear trade.

It is of interest also to compare the toughness curves of the varieties shown in Figure 2 with those of the dent varieties, Boone County White and Reid Yellow Dent, which were presented in a previous paper,[8] and which show that these two varieties were also relatively tender up to 30 days of age. It is by no means certain that all flint, flour, and waxy varieties are extremely tough or that all dents are relatively tender, even though records so far obtained indicate that this is the case.

The significance of the results recorded in Figure 2 will be discussed in another section of this article.

CHEMICAL STUDIES

The chemical constituents of corn, their nature, relative proportions, and physical conditions within the kernel, determine to a very large degree the suitability of any corn for canning purposes. This is particularly true of the carbohydrates. Within this group of substances are to be found those which determine

8 CULPEPPER, C. W., and MAGOON, C. A. Op. cit.
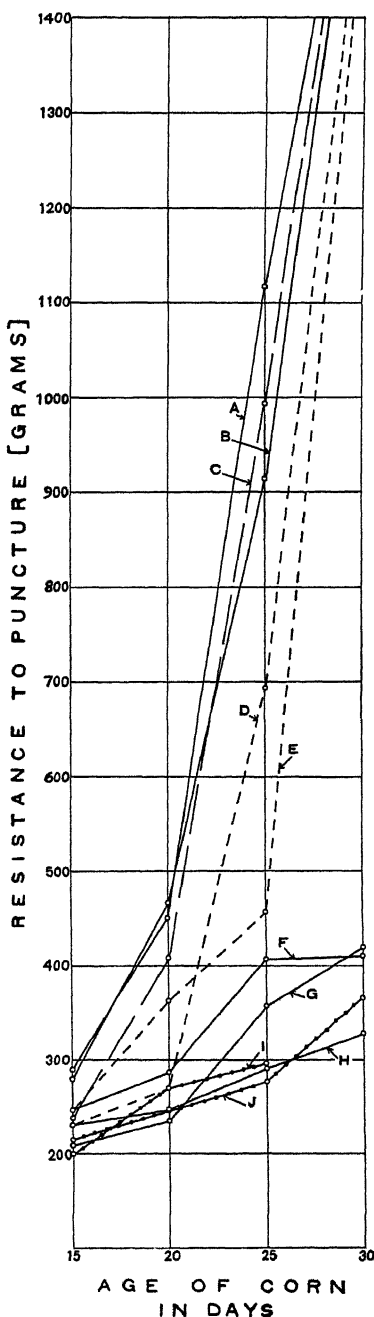
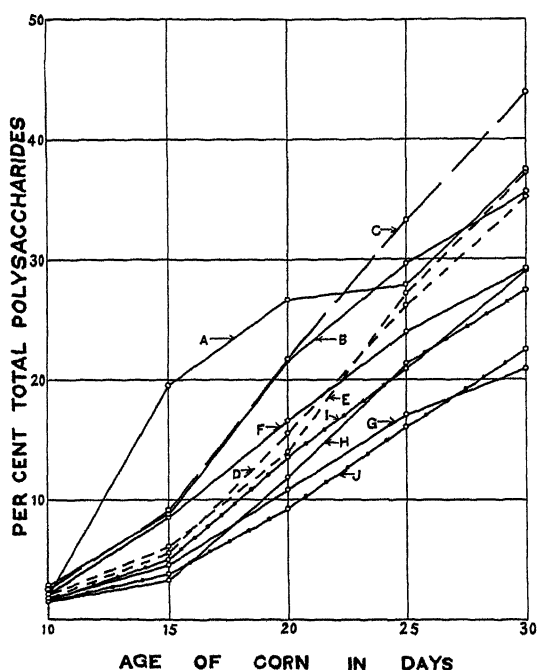FIG. 2.—Degree and rate of increase in toughness of corn of different types and varieties with advancing maturity: A, Mandan White; B, Yellow Assiniboine; C, Waxy maize; D, Rhode Island White; E, Longfellow; F, Guatemalan; G, Stowell's Evergreen; H, Golden Bantam; I, Second Early Adams; J, Boone County White (from data collected in 1922 and introduced here for comparison)

the natural sweetness of the corn and the consistency and texture of the canned product.

The rôle of the nitrogenous constituents and of the oil of corn in their influence on quality is not known. It is possible that some of the distinctive flavors of corn of the different types and of corn at different stages of maturity are more or less closely associated with these substances. No investigation seems to have been made to determine such a relationship. The experiments covered by this paper did not include such a study. The presence of the distinctive fresh corn flavors and their rate of disappearance in the harvested corn seem to parallel closely the changes in sugar content.

The analytical data here presented, therefore, are concerned primarily with the carbohydrates of corn of the different types under investigation and the changes taking place in them as maturity advances.

It is a common notion that the property of sweetness distinguishes the so-called sweet corns from other types, making them better adapted to canning purposes, and that the accumulation of starch in the kernel is what gives to the corn in the can its desirable creamy consistency and texture. It has been shown by the writers,[9] however, that a relatively high sugar content is not confined to the sweet corns alone, and that the presence of dextrin or dextrinlike compounds in the sweet corns has much to do with the desirable physical properties of their canned product.

Among the different types of corn selected for the experiments here discussed a wide range of conditions, as regards the nature and relative proportions of the different carbohydrates, is known to exist. Waxy maize, for instance, has a very high dextrin content; the flint, flour, and dent varieties not only have high starch contents but the physical condition of the starch in the kernel differs greatly; and among the different types a considerable range in sugar content is found.

It is of interest, therefore, to follow the chemical transformations occurring in these different corns and to correlate the findings with those of practical canning tests. The analytical data are presented in Table 2 and are illustrated graphically in Figures 3 to 6, inclusive.

In the consideration of these figures and the following discussion of them it must be borne in mind that only a few varieties of the different types of corn have been included in the present experiments. The conclusions drawn from these results, therefore, may be found untenable when a larger number of varieties have been studied.

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TABLE 2.— *Percentage composition of corn of different types and varieties at different stages at maturity*

Type or variety	Stage of maturity in days	Total solids	Alcoholic extract	Residue	Sugars Total Fresh-weight basis	Total Moisture-free basis	Reducing Fresh-weight basis	Reducing Moisture-free basis	Sucrose Fresh-weight basis	Sucrose Moisture-free basis	Polysaccharides Total Fresh-weight basis	Total Moisture-free basis	Water-soluble Fresh-weight basis	Water-soluble Moisture-free basis
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Mandan White (flour)	5	11.68	6.68	5.00	4.87	41.70	3.55	30.40	1.32	11.30	2.16	18.49	0.21	1.80
	10	11.33	6.64	4.69	5.10	45.01	3.00	26.47	2.10	18.54	1.91	16.85	.21	1.85
	15	22.57	6.96	15.61	4.35	19.27	1.25	5.53	3.10	13.74	10.96	48.56	2.17	9 61
	20	37.68	5.62	32.06	3.08	8.17	.53	1.40	2.55	6.77	25.17	66.80	2.00	5.30
	25	44.31	4.72	39.59	1.56	3.96	.19	.48	1.37	3.48	27 91	71.00	1.28	3.25
	30	52.40	3.28	49.12	1.25	2.38	15	.28	1.10	2.10	37.43	71.43	1.86	3.55
Yellow Assiniboine (flour)	5	12.35	7.84	4.51	5.40	43.72	4.02	32.55	1.38	11.17	1.89	15.30	.13	1.05
	10	12.18	7.12	5 06	5.08	41.54	2.76	22.06	2.32	18.88	2.08	17.07	.18	1.47
	15	21.97	7.72	14.25	5.20	23.66	1.41	6.41	3.79	17.25	8.90	40.51	1.13	5.14
	20	34.86	6.32	28.54	2.91	8.34	.59	1.69	2.32	6.65	21.66	62.13	1.22	3.50
	25	42.65	4.32	38.33	1.45	3.40	.19	.44	1.26	2.96	29.66	69.56	1 61	3.77
	30	48.75	3.92	44.83	1.35	2.76	.12	.24	1.23	2.52	35.06	71.92	1.38	2.83
Waxy maize	5	10.96	6.12	4.84	4.12	37.59	3.43	31.29	.69	6.30	2.12	19.34	.15	1.36
	10	12.95	7.40	5.55	5.31	41.00	3.58	27.79	1.73	13.21	2.41	18.61	.30	2.31
	15	22.33	8.26	14.07	5.51	24.67	2.17	9.71	3.34	14.96	9.10	40.75	2.11	9.44
	20	35.04	7.00	28.04	3.86	11.01	1.04	2.96	2.82	8.05	21.62	61.70	8.29	23.65
	25	47.82	5.98	41.84	2.55	5.33	.37	.77	2.18	4.56	33.26	69.55	3.97	8.30
	30	55.76	4.10	51.66	1.80	3.22	.24	.43	1.56	2.79	40.39	72.55	5.47	9.81
Rhode Island White (flint)	5	13.18	7.10	6.08	5.10	38.69	3.68	27.99	1.42	10.70	3.20	24.28	.25	1.89
	10	11.06	6.64	4 42	4.37	39.51	3.32	30.02	1.05	9.49	1.96	17.72	.21	1.90
	15	17.08	7.94	9.14	5.45	31.90	2.00	11.71	3.45	20.19	5.53	32.37	1.03	6.03
	20	27.86	6.56	21.30	3.44	12 34	.94	3.37	2.50	8.97	15.07	54.09	2.12	7.61
	25	40.60	7.10	33.50	3.20	7.88	.60	1.47	2.60	6.41	26.26	64 68	3.41	8.40
	30	51.00	4.82	46.18	2 24	4.39	.37	.72	1.87	3.67	35.14	68.90	2.86	5.61
Longfellow (flint)	5	12.43	6.66	5.77	4.46	35.88	3.25	26.14	1.21	9.74	3.15	25.34	.22	1.78
	10	11.68	6.32	5.36	4.16	35.61	3.06	26.19	1.10	9.42	2.38	20 37	.20	1.71
	15	16.87	6.94	'9.93	4.19	24 83	1.67	9.89	2.52	14.94	6.11	36.21	.64	3.80
	20	25.27	6.96	18.31	3.82	15.11	1.09	4.31	2.73	10.80	14.00	55.40	2 37	9.37
	25	40.90	5.64	35.26	2 73	6.67	.47	1.15	2.26	5.52	27.18	66.45	3.06	7.48
	30	54.02	4.72	49.30	1.79	3.31	.17	.31	1.62	3.00	37.31	69.06	2.56	4.73
Second Early Adams (dent)	5	11.25	6.20	5.05	4.16	37.00	3.02	26.84	1 14	10.16	2.37	20.17	.17	1.51
	10	10.56	6.44	4.12	4.31	40.81	3.24	30.68	1.07	10.13	1.79	16.03	.13	1.23
	15	18.37	7.64	10.73	5.16	28.08	2.45	13.33	2.71	14.75	5.16	28.09	.83	4.51
	20	23.91	5.72	18 19	3 05	12.75	.76	3.17	2.29	9.58	13.73	57.38	2.16	9.03
	25	32.27	5.48	26.79	2.41	7.46	.46	1.42	1.95	6.04	21.05	65.23	1.25	3.87
	30	42.52	4.86	37.66	1.86	4.37	.23	.54	1.63	3.83	29.07	68.36	1.82	4.27
Guatemalan (sweet)	5	9.94	5.42	4.52	4.12	41.44	3 11	31.28	1 01	10.16	1.77	17.80	.15	1.51
	10	11.23	6.08	5.15	5.31	47.28	2.56	22.80	2.75	24.48	2.15	19.14	.23	2.05
	15	20.27	7.28	12.99	5.51	27.18	1.36	6.71	4.15	20.47	8.57	42.28	2.90	14.30
	20	29.39	6.84	22.55	3.82	13.00	.81	2.75	3.01	10.25	16.57	56.38	7.86	26.74
	25	36.82	6.00	30.82	2 55	6.92	.49	1.33	2.06	5.59	24.04	65.29	11.52	31.28
	30	44.26	4.86	39.40	1.80	4.06	.30	.67	1.50	3.39	29.27	65.90	12.60	28.46
Stowell's Evergreen (sweet)	5													
	10	10.84	6.30	4.54	4.39	40 50	2.78	25.64	1.61	14.86	1.86	17.16	.58	5.35
	15	16.05	8.32	7.73	6 51	40.56	1.94	12.08	4.57	28.48	4.69	29.22	1.85	11.52
	20	22.05	7.22	14.83	4.45	20.18	.84	3.80	3.61	16.38	10.90	49.07	6.67	30.24
	25	27.76	5.68	22 08	3.06	11.02	.62	2.23	2.44	8.79	17.13	61.70	9.27	33.39
	30	32.18	5.46	26.72	2 78	8.63	.63	1.95	2.15	6.68	20.89	64.91	12.18	37.84
Golden Bantam (sweet)	5	10.08	5.72	4.36	3.74	37.10	2.98	29.56	.76	7.54	1.43	14.18	.11	1.09
	10	10.14	5.98	4 16	3.95	38.95	3.14	30 96	.81	7.99	1.52	15.00	.11	1.08
	15	16.15	9.16	6.99	6.91	42.78	2.43	15.04	4.48	27.74	3.37	20.86	1.18	7.30
	20	24.24	7.98	16.26	5.45	22.49	1.38	5.69	4.07	16.80	11.96	49.34	6.83	28.17
	25	34.36	6.52	27.84	3.35	9.75	.59	1.71	2.76	8.04	21.38	62.28	11.67	33.96
	30	41.27	4.20	37.07	1.31	3.17	.37	.89	.94	2.28	27.43	66.49	12.67	30.70

FLOUR CORNS

The two varieties of flour corn used in these tests proved to be early, reaching the silking peak about 53 days from the date of planting and maturing very rapidly after pollination had taken place. It was unfortunate that a late variety was not included in this group.
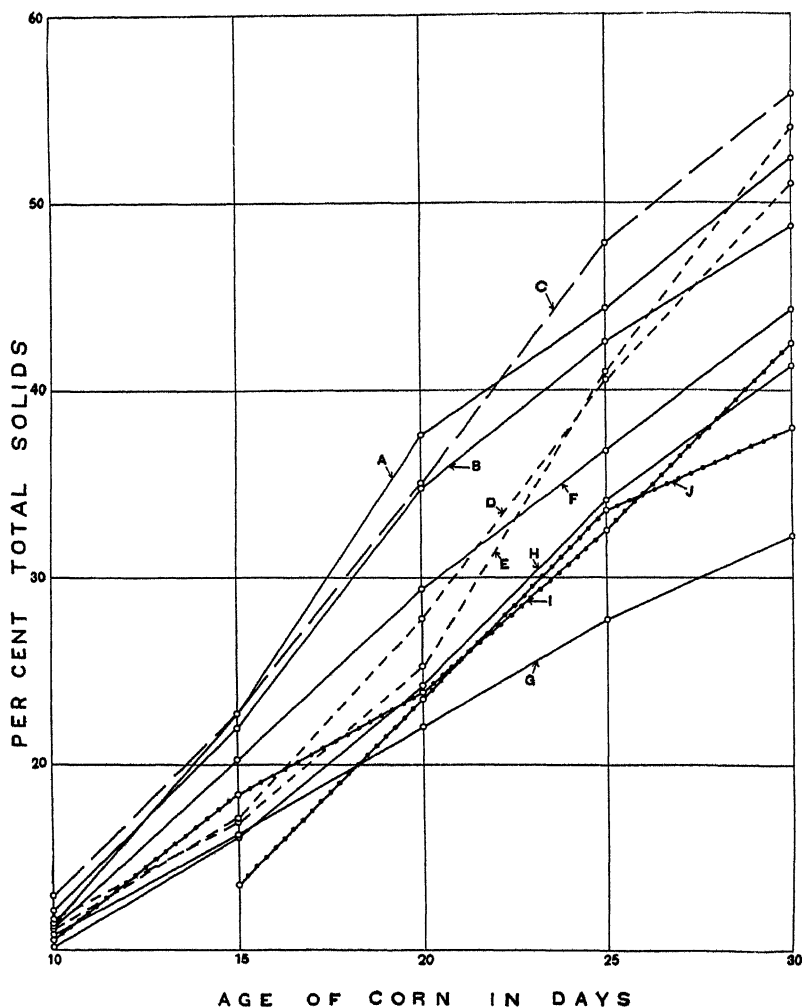
FIG. 3.—Degree and rate of increase in total solids in corn of different types and varieties with advancing maturity: A, Mandan White; B, Yellow Assiniboine; C, Wavy maize; D, Rhode Island White; E, Longfellow; F, Guatemalan: G, Stowell's Evergreen, H, Golden Bantam; I, Second Early Adams; J, Boone County White (from data collected in 1922 and introduced here for comparison)

As already pointed out, the flour type of corn is characterized by a soft, starchy endosperm, the cornaceous or flinty portion being very thin and inconspicuous. The floury character of the kernel contents, according to Weatherwax,[10] is due to the relatively small amount of

[10] WEATHERWAX, P. THE STORY OF THE MAIZE PLANT. 247 p., illus. Chicago, Ill. [1923].

protein or colloidal carbohydrate occupying the interspaces between the starch grains. The size and shape of the starch grains may also be in part responsible for the condition.

Examination of the analytical data shows that with respect to moisture content at the different stages of maturity the flour corns were lower than all the other varieties studied, except the flint corns at the 30-day stage, and the waxy maize, which the flour corns paralleled during the first 20 days of development and then exceeded in the later stages. The higher moisture content in the 25 and 30 day corn was due probably to the failure of the kernels to become as compactly filled as in the case of the waxy type.

The sugar content of the flour corns was lower than in the sweet corns, although the maximum attained was above 5 per cent. The decrease in amount was rapid after the 15-day stage and in the 25
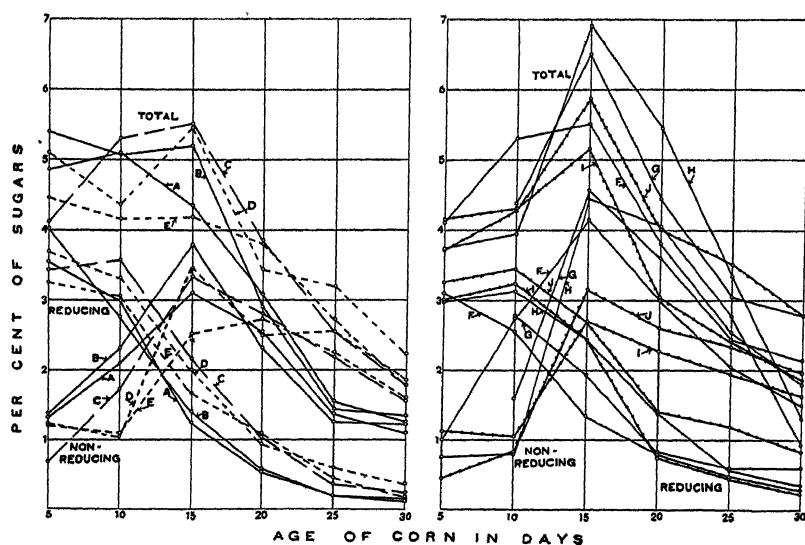
FIG. 4.—Sugar content of corn of different types and varieties at different stages of maturity: A, Mandan White; B, Yellow Assiniboine; C, Waxy maize; D, Rhode Island White; E, Long-fellow; F, Guatemalan; G, Stowell's Evergreen; H, Golden Bantam; I, Second Early Adams; J, Boone County White (from data collected in 1922 and introduced here for comparison)

and 30 day corn the sugar content was lower than in any of the other varieties studied.

The water-soluble polysaccharides were very small in amount and did not increase significantly in the corn beyond the 15-day stage. The very low content of water-soluble polysaccharides in the later stages is particularly significant and helps to explain the floury character of the endosperm.

The percentage of total polysaccharides present in corn is determined to a large degree by the moisture content. The polysaccharides increase as the moisture content decreases and are less abundant where the moisture content is high. In the present case the proportion of polysaccharides also increased as the kernels became more mature. Considered from the moisture-free basis, the total polysaccharide content of these flour corns during the later stages was greater than in any of the varieties studied except waxy maize. This furnishes in part a chemical basis for their floury character.

WAXY MAIZE

Only one variety of waxy maize was available for these studies. Waxy maize is a very distinct type of corn, resembling in general form and appearance some of the flint varieties. The endosperm, however, on breaking has a waxlike appearance, which has given rise to the name applied to this type of corn. The waxy character applies to physical appearance only, the polysaccharide of which it is principally composed being erythro-dextrin, according to Weatherwax.[11]

This was the latest of the varieties studied. It required 96 days to reach the peak of silking and remained green for a considerable period.

FIG. 5.—Degree and rate of increase in total polysaccharides in corn of different varieties with advancing maturity: A, Mandan White; B, Yellow Assiniboine, C, Waxy maize; D, Rhode Island White; E, Longfellow; F, Guatemalan; G, Stowell's Evergreen; H, Golden Bantam; I, Second Early Adams; J, Boone County White (from data collected in 1922 and introduced here for comparison)

The analytical data show a low moisture content throughout the entire period of development and maturing of the kernel. The moisture decreased continuously and rapidly up to 30 days from silking, when the amount present was slightly less than 45 per cent.

The polysaccharide total was very high, corresponding to the low moisture content, and consisted almost entirely of dextrin, as indicated by the iodine test. Its solubility, however, was entirely different from the ordinary dextrin, as a large proportion of it was insoluble in cold water. The water-soluble portion in the early stages of development was about the same or slightly greater in amount than in the sweet corns. In the waxy maize 25 and 30 days old, however, the proportion was very much less than in the sweet corns. This is very clearly shown in Figure 6.

The sugar content of waxy maize was somewhat lower than in Stowell's Evergreen and Golden Bantam, about equal to that of the flint varieties, and somewhat higher than in the flour corns and the dent variety, Second Early Adams. The total sugar content amounted to about 5½ per cent in the corn at the 15-day stage, but the ratio of reducing sugars to sucrose was high, indicating for the corn a degree of sweetness somewhat lower than the percentage of total sugars would ordinarily signify.

[11] WEATHERWAX, P. Op. cit.

FLINT CORNS

The two flint varieties used in these tests were typical of the New England grown corn of the flinty type, the hard, full, and well-rounded kernels of the mature corn being borne on long slender ears. In field behavior and in general appearance of the ears the two varieties tested were similar, differing only in the color of the grains.

These flint corns were medium late in maturing. It required about 73 days after planting for each to reach the silking peak.

According to Weatherwax,[12] the flinty or horny character of the endosperm in the flint corns is due to the presence in the cells of protein and colloidal carbohydrates which completely fill the interspaces between the starch grains.

The chemical analyses show that the flint corns in this case had a rather high moisture content during the early stages of development. As maturity advanced, however, the moisture content fell off rapidly, the corn 30 days old averaging lower in moisture even than the flour corns, and only about 3.2 per cent higher than waxy maize.

Figures 2, 3, and 4 indicate that during the early stages the flint corns developed somewhat more slowly than the flour corns and waxy maize, and therefore had a higher moisture content.

Fig 6.—Variation in degree of water-soluble polysaccharides in corn of different types and varieties at different stages of maturity: A, Mandan White; B, Yellow Assiniboine; C, Waxy maize; D, Rhode Island White; E, Longfellow; F, Guatemalan; G, Stowell's Evergreen; H, Golden Bantam; I, Second Early Adams, J, Boone County White (from data collected in 1922 and introduced here for comparison)

The flint corns developed a somewhat greater proportion of water-soluble polysaccharides than the flour types, especially in the later stages of development, but they were far below the types of sweet corn in this respect. This is clearly shown in Figure 6. During the later stages of development the total polysaccharide content was higher in flint corns than in the sweet types, and averaged about

[12] WEATHERWAX, P. Op. cit.

the same as the flour types at the 30-day stage of maturity. In sugar content the flint corns were considerably lower than the sweet corns but higher than the flour corns, especially as maturity approached.

DENT CORNS

In the physical structure of the kernel the dent corns are intermediate between the flint and the flour types, the endosperm, as earlier pointed out, having two distinct portions, an outer horny or flinty portion and a central floury core extending to the tip of the grain. The shrinkage of this portion at the tip of the kernel as it dries out is what gives to it its dented form.

Only one representative of this group, Second Early Adams, was grown in the present case. This is a very early corn, requiring, under the present conditions, but 55 days after planting to reach the peak of silking. In this respect it compares favorably with Golden Bantam. Its earliness, tenderness, and other attractive qualities make it a favorite among truck-crop growers for the fresh-corn market.

In moisture content and in total polysaccharides, also, the Second Early Adams resembled Golden Bantam. In the proportion of water-soluble polysaccharides, however, this variety more closely resembled the flour corns, the amount present being small.

While a maximum total sugar content of a little over 5 per cent developed in the corn 15 days old, nearly one-half of the entire amount consisted of reducing sugars, indicating a corn low in sweetness. The sucrose present, however, did not disappear as rapidly as in the sweet varieties or the flour corns as maturity approached. In this respect Second Early Adams behaved like the flint and the waxy corns.

For the sake of a better understanding of the behavior of dent corns in general during the development and maturing of the kernel, reference is made to the results of similar tests upon two other dent varieties, Boone County White and Reid Yellow Dent, recorded in the earlier paper already mentioned.[13] While the percentages vary somewhat, the results obtained for these varieties show the same general trend as those given here for Second Early Adams. In the charts accompanying this discussion curves illustrating the findings on Boone County White in this earlier work have been included for comparison.

SWEET CORNS

The physical characteristics of the two sweet corns, Golden Bantam and Stowell's Evergreen, are too well known to require detailed consideration here. The story of their chemical behavior during the development and maturing of the kernel has already been told.[14] Under the conditions of the present season Golden Bantam required 54 days after planting to reach the silking peak, while Stowell's Evergreen, planted the same day, required 66 days to reach the same stage of development.

A third variety, which for lack of a definite name has been termed here "Guatemalan," was included in this group because it was known to be one of the very latest of the sweet type. In this case it required 92 days to reach the peak of silking.

The moisture content of these different varieties is of particular interest to the writers. In the earlier study, Golden Bantam, an early

[13] CULPEPPER, C. W., and MAGOON, C. A. Op. cit.
[14] MAGOON, C. A., and CULPEPPER, C. W. Op. cit.

variety, was found to have a relatively low moisture content, and Stowell's Evergreen, a late variety, was found to have a very high moisture content. The conclusion drawn from these earlier studies was that a close correlation exists between the moisture content and rate of maturing of the corn. The present study has confirmed the previous conclusions with regard to Golden Bantam and Stowell's Evergreen. When Guatemalan sweet was analyzed, however, it was found that instead of having a moisture content higher than that of Stowell's Evergreen, which required 66 days to reach the silking peak, its moisture content was actually considerably lower than that found in Golden Bantam, which required 54 days to reach the silking peak. Since, as already mentioned, Guatemalan required 92 days to reach the peak of silking, it is evident that the moisture content is a specific characteristic of the variety and is more or less independent of the rate at which the corn matures.

As a group, the sweet corns, together with the dent corn, had a relatively high moisture content, particularly in the later stages of maturity.

The outstanding features in the chemical composition of the sweet corns were a relatively high sugar content and a high percentage of water-soluble polysaccharides, consisting of dextrin and soluble starch. In the case of the Guatemalan sweet the maximum sugar content was 5½ per cent, which point was also equaled or approached by several of the nonsweet corns; but the percentage of sucrose in Guatemalan was higher than in any of these.

With but one exception—that of the Guatemalan sweet in its early stages of development—the sweet corns were lower in total polysaccharides throughout their entire development than the flour, flint, and waxy corns.

Taking into consideration these carbohydrate relationships, it would appear that among the flour, flint, and waxy corns there is a greater tendency for the sugars to be completely converted into starch than in the sweet and dent corns. On the other hand, the metabolic processes by which the sugars are converted into starch are partially arrested in the last-mentioned types, permitting an accumulation of sugar, but resulting ultimately in a lower polysaccharide content. In the flints and in the horny portions of the dent corns the starch grains seem to be embedded in a matrix consisting largely of soluble starch, while in the flour corns and the floury portion of the dent endosperm this matrix seems to be almost entirely absent. In the sweet corns a large amount of dextrin and soluble starch forms the matrix for the relatively few starch grains. This gives to the sweet-corn kernel its translucent endosperm. It is the loss of water by the endosperm in drying that causes the wrinkling of the grain.

CANNING TESTS

In order to observe the effect of the physical and chemical properties of corn of the types herein discussed on the quality of the canned corn, sample lots of ears were harvested from the different varieties, at the 20-day stage of maturity, and sample packs prepared from each. The canning methods employed were the same for all varieties, so that the canned products were strictly comparable. At the end of the season and at irregular intervals thereafter sample cans from the various lots were opened and the products subjected to careful comparative study. The results of these tests are presented below.

SWEET CORNS

The canned products from the Golden Bantam and Stowell's Evergreen were characteristic for these varieties and did not differ from those previously described.[15, 16] They were tender, of good texture and creamy consistency, sweet, and well flavored. They easily graded as first-quality products.

The canned corn from the Guatemalan sweet was somewhat poorer in quality. It was not so tender, the consistency was heavier, and it was less sweet. It was apparent that toughness was responsible to a considerable extent for the poorer product obtained from this variety.

These findings were in accord with the results of the chemical analyses and the earlier tests for toughness. Figure 2 shows that the puncture-test readings for Guatemalan sweet were considerably above those for Golden Bantam and Stowell's Evergreen. Table 2 and Figures 3 and 4, on the other hand, indicate that Guatemalan was lower in moisture content and in sugar than either of the other varieties. The puncture tests on the raw corn provided an accurate index of the tenderness of the corn in the can, while the moisture content was reflected directly in the consistency of the canned product.

FLOUR CORNS

The canned products from the two varieties of flour corns were similar in all respects. They were hard and tough, dry, and lacking in sweetness, and instead of the desirable flavor characteristic of sweet corn there was a hominylike taste which detracted from the quality. The creamy consistency observed in the products from the sweet varieties was entirely absent from the products of the flour types. These corns, therefore, were found to be wholly unsuited for canning purposes.

The two features most seriously affecting the quality in the flour corns were the extreme toughness of the kernel hulls and the hard, lumpy character of the kernel contents. Figure 2 shows that the pronounced toughness of the pericarp was foretold in the puncture tests, while a comparison of the chemical data on polysaccharides shows the reason for the hard and lumpy character of the product. In the case of the sweet varieties approximately one-half of the total polysaccharides were soluble in cold water. These gave to the canned product a creamy character, whereas in the flour corns only small quantities of water-soluble polysaccharides were present, the bulk of these being composed of starch which merely swelled during the canning process.

Water-soluble polysaccharides play a very important part in determining the quality of canned corn.

WAXY CORN

The canned product from waxy corn was likewise of poor quality. The kernel hulls were very tough, which detracted materially from the attractiveness of the corn. The product was rather dry in consistency. The kernel contents, while somewhat hard, were waxlike or

[15] CULPEPPER, C. W., and MAGOON, C. A. Op. cit.
[16] MAGOON, C. A., and CULPEPPER, C. W. Op. cit.

gummy rather than starchy in character. The corn was fairly sweet, but there was no distinctive flavor. This type of corn was decidedly unsuited for canning.

These findings have their confirmation and explanation in the physical and chemical data already presented. The puncture tests showed this variety to have a very tough pericarp. The low moisture content found accounts for the heavy consistency, while the mixture of nonsoluble and water-soluble dextrins explains the peculiar waxlike texture of the product.

FLINT CORNS

The canned material from the flint varieties, although not quite so dry, resembled in physical appearance the product from the flour corns. The hulls present were tough and the kernel contents hard and lumpy. It was lacking somewhat in sweetness, but the flavor was superior to that of flour corn. On the whole, the flint-corn product graded slightly higher than that from the flour corns but was far inferior to the product from the sweet varieties. The most objectionable features were the tough hulls and the hard kernel contents.

DENT CORN

The Second Early Adams was much more tender than any of the other field varieties. This tenderness was reflected in the quality of the canned product. In this respect it closely approached the sweet corns. The flavor and sweetness of the product were also very satisfactory. It lacked the smooth texture and creamy consistency characteristic of sweet corn, however, and the rather lumpy character of the kernel contents detracted from the quality of the canned material.

These findings agree well with those resulting from the puncture tests and the chemical analyses. The lack of creaminess finds its explanation in the low percentage of water-soluble polysaccharides in dent corn.

DISCUSSION

In the present work various types of corn have been studied and the possibility of their use for canning purposes has been discussed. The object of this investigation, however, was not so much to determine the merits of the different types for canning as to make through them an analysis of the factors which constitute what is termed "quality" in canned corn.

Two groups of factors influence quality in canned corn. One has to do with harvesting methods and cannery practices, and the other with the natural properties of the corn itself. It is the last-named factor with which this investigation is concerned. Of the factors included in this group may be mentioned the toughness of the pericarp, the consistency or creaminess of the kernel contents, the sugar content or sweetness, the moisture content, the compactness of the insoluble material in the endosperm, and the flavor.

The relative importance of any factor depends upon the degree of variation which occurs, the readiness with which it may be corrected by methods of handling, and the way it is affected by other existing factors. Thus, if sugar content is the only significantly variable factor, the variety having the highest sugar content is the most desirable; and if the outstanding variation is in the tenderness of the pericarp, that corn in which it is toughest is the least desirable.

A study of the types here discussed shows that the tenderness of the kernels varies widely, both with the degree of maturity and with the type of corn, as well as with the varieties within the types. The sugar content is quite variable in the different types and in the varieties within the types. It varies much more widely, however, with the degree of maturity. The texture and consistency of the canned product also is subject to considerable variation, the former with the type of corn used and the latter both with the type and the degree of maturity. Flavor is much less variable. Significant variations do occur, but the differences seem less objectionable than variations in tenderness. The significance of these factors will be discussed in greater detail in the following paragraphs.

TENDERNESS

The results of the experimental work reported above indicate clearly that of the inherent properties which affect the quality of canned corn, the degree of tenderness of the pericarp is most important. In measuring this degree of tenderness or toughness by mechanical means some slight error is involved because of the structure of the underlying tissues, which give support to the kernel hull. When the kernels are compactly filled with starch and dextrin, as in the corn at the more mature stages, the readings are correspondingly high. This is not an important consideration, however, for experience has shown that the puncture-test readings furnish a very reliable working index of the probable tenderness of the cut-out corn.

The quantity of the pericarp entering into the canned product has an effect on the apparent toughness or age of the corn. Thus, corn that is a little tough will appear more tender and less mature if it is double cut or slit and scraped than when canned in the so-called "Maryland style," where the kernels are practically whole.

The toughness of the pericarp is not noticeably affected by an increase in the processing period. Tough corn could not be made tender even if the objectionable features of overprocessing could be surmounted.

In general the field varieties are considerably tougher than the sweet varieties at comparable stages of maturity. This in large measure accounts for their being unsuited for canning purposes. The dent varieties used in these and preceding tests were found to be more tender than the other field types. Second Early Adams, belonging to this group, was about as satisfactory from this standpoint as Golden Bantam or either of the other sweet varieties.

The age of the corn, or its degree of maturity, determines very largely the degree of toughness of the kernel hull. The toughness increases steadily and, in many cases, rapidly as maturity approaches. Were it not for the fact that other factors besides toughness have to be taken into consideration in the grading of corn for canning, it could be harvested when young and tender. In very young corn, however, those constituents which give proper body or consistency to the canned product are lacking. Too often, therefore, tenderness is sacrificed to consistency.

It seems evident that the most satisfactory way to meet the requirements for tenderness is to can the corn at as early an age as the consistency of the kernel contents will permit, and to endeavor by selection and breeding to develop tenderer strains, so that more mature corn may be used without sacrifice of quality.

TEXTURE AND CONSISTENCY

Although the body or consistency of the kernel contents usually determines when the corn shall be harvested, the experiences of the present work have forced the conclusion that this factor holds second place in determining quality.

Two elements enter into the determination of consistency—the nature of the polysaccharides present and the moisture content. It will be recalled that about one-half of the total polysaccharides present in sweet corns at canning maturity are water-soluble, being in the form of dextrin or dextrinlike compounds. These are responsible for the creamy texture of the canned product and when combined with the proper amount of moisture give the desired body to the canned material. In order to get the proper consistency it is always necessary to add liquid to the cut-off corn. If too little liquid is added or the corn is too mature the finished product will be too dry or viscous. Regardless of how much or how little liquid is added to it, the canned product of very young corn will not be creamy, because sufficient polysaccharides have not been stored in the kernels.

The water-soluble polysaccharides are practically absent in all field varieties except the waxy corn. On account of the presence of starchy lumps the canned product from these varieties is more or less granular and the creamy texture is entirely lacking. While considerable quantities of water-soluble polysaccharides are present in the waxy maize, the remaining carbohydrate is so compactly stored in the kernel that the texture and consistency are unsatisfactory.

The consistency of canned corn is modified greatly by the method of cutting and by precooking. The fineness with which the corn is cut affects both the appearance of the pack and its consistency, and the product is given a smoother and more creamy texture by the agitation given to the corn in the precooker.

In Maryland-style corn where the kernels are removed from the ear nearly whole, a viscous or cloudy liquor is objectionable and consistency is of no consideration. "Blanching" corn on the cob prior to cutting, as is sometimes practiced by home canners, yields a product that is less creamy. This is accounted for primarily by the fact that the kernel contents are coagulated by the heat and less of the material passes out into the added liquid.

SUGAR CONTENT

Natural sweetness is next in order of importance among the factors affecting quality in canned corn. The sweetness is roughly proportional to the total quantity of sugar present. The quantity and relative proportions of the different groups of sugars in corn are constantly changing as development proceeds. At the time corn is ordinarily canned, sucrose, or cane sugar, is responsible for most of its natural sweetness. The ease with which deficiency in sweetness may be remedied by the addition of sucrose in the brine has raised some question as to whether natural sweetness is of much significance in corn. Sweet corns, as a rule, contain more sugar than the field varieties. In some cases, however, the difference is not great. Occasionally, also, where the sugar content is relatively low, other desirable features, such as tenderness, for instance, make the corn more acceptable than others having a higher sugar content. The present study indicates that natural sweetness is not often the deciding factor in the choice of the best type or the best variety of corn.

Corn containing 5½ per cent of sugar, or approximately the concentration normally present in corn at its highest sugar content point, is considered most desirable for the canned product. When corn is ready for canning it has passed somewhat beyond its highest point of sweetness, therefore it is necessary to add sucrose at the time of canning in order to obtain the most acceptable product. The quantity varies from 4 to 10 per cent in the brine, depending on the condition of the corn. The average brine contains about 6¼ per cent of sugar.

It is the opinion of some that added sugar fails to supply the quality of sweetness produced by the natural sugar of the corn. Lack of sugar, however, is nearly always associated with other objectionable properties in the corn, such as toughness, too heavy consistency, and poor flavor, so that it is questionable whether this opinion has much weight.

Since the percentage of sugar in any variety is constantly changing, it might be supposed that the stage of maturity at which the sugar content was highest would be optimum for canning. This is not the case. In the region of Washington, D. C., for instance, corn reaches its highest sugar content about 15 days after the first appearance of silks; but at this stage the corn, particularly of the late varieties, is much too immature to give desirable body and flavor.

High sugar content is desirable in corn for canning, and contributes much to the quality of the canned product,. Other factors, however, appear to be of considerably more importance.

FLAVOR

Sweetness in corn constitutes much of what the average person considers flavor. Experiments show that corn in prime canning condition which is canned without sugar lacks the flavor of corn canned with sugar. High sugar content, however, does not always mean high flavor or quality. In the present tests corn 15 days old from silk, though higher in sugar content than others more mature, was considered by nearly all judges as lacking in flavor. Just what chemical constituents are responsible for the pleasing characteristic flavor of prime sweet corn is not known.

Very small differences in flavor occur in varieties of the sweet type at comparable stages of maturity, such differences being more particularly marked between the white and the yellow varieties. The variations in flavors among the different types of corn are somewhat greater. Only in rare instances, however, are even these type differences considered of much importance.

As corn approaches maturity the pleasing flavor gradually disappears and is replaced by one considerably less attractive.

COMPACTNESS OF THE ENDOSPERM

As already suggested, a part of what is usually considered toughness is really due to the compactness of the endosperm. In the flint corns, and in the waxy type especially, the endosperm is very hard and compactly filled with carbohydrate. This becomes especially marked as the corn approaches maturity. The endosperm of the sweet varieties is much less compact than that of any other type. There is considerable variation among the sweet corns in this respect, as was shown by the Guatemalan sweet and Stowell's Ever-

green in the present tests.   The kernels of the Guatemalan sweet were considerably more compactly filled than Stowell's Evergreen, as shown not only by physical appearance but also by their lower moisture content.

As affecting the quality of the product from the varieties usually canned this factor probably is not of much significance.

CELLULAR STRUCTURE

No histological studies were made of the different types of corn used in these tests.   The readiness with which the contents of the kernels of some of the sweet varieties escaped into the liquid during the canning process, however, suggests that there may be some marked difference in the cellular structure.   Size of the cells or thickness of the cell walls may vary considerably in the different types and varieties and may have some influence on the quality of the canned material obtained from the different corns.

SUMMARY

Several types of maize have been studied with respect to the degree of toughness of the pericarp, the chemical composition of the kernels, and the canning quality of the different types.

There is great variation in the different types and in all types at different stages of maturity in the toughness of the pericarp.   The degree of toughness is much greater in the flour, flint, and waxy types than in the dent and sweet corns.   This toughness is particularly marked with the approach of complete maturity.

The most characteristic difference in chemical composition is found in the content of water-soluble polysaccharides.   The sweet corns have a very high proportion of this constituent.   It is relatively high also in the waxy maize but rather low in the flint, dent, and flour types.

There is a very significant difference in the various types as well as in varieties of the same type in the moisture content of the developing kernels.   This difference generally becomes more marked as maturity approaches.

In general, the sugar content is higher in the sweet varieties than in the other types, but the differences are not so marked as might be expected, in view of the preference generally shown to sweet corn for table purposes.

In tests upon the relative quality of the different types for canning purposes the sweet corns were found superior to all other types, followed in order by the dent, waxy, flint, and flour types.

From a study of the data here presented it is possible to list the following as factors which are preeminent in determining the quality of the canned product:

1. The degree of tenderness or toughness of the pericarp.
2. The nature of the polysaccharides present, and the ratio of water-soluble to total polysaccharides.
3. The sugar content.
4. The compactness with which the polysaccharides are laid down in the endosperm, and possibly the cellular structure of the endosperm itself.

RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE TO THE INFECTION OF SWEET POTATOES BY THE STEM-ROT ORGANISMS [1]

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INTRODUCTION

The relation of soil temperature and soil moisture to the infection of different crops by parasitic fungi has been investigated by a number of workers. Monteith [2] showed that soil temperature was not a limiting factor in the infection of cabbage by *Plasmodiophora brassicae* Wor., since infection occurred at widely different temperatures. Soil moisture, on the other hand, was found to be important. Gilman [3] showed that infection of cabbage by *Fusarium conglutinans* Woll., the organism causing yellows, occurred only at relatively high temperatures, which would indicate that the most severe development of the disease might be expected during seasons in which high temperatures prevailed in the early part of the growing period. Jones, McKinney, and Fellows [4] found that infection of potatoes by the scab organism, *Actinomyces scabies* (Thaxt.) Güssow, is favored by relatively high temperatures, and Tisdale [5] demonstrated that there is a close correlation between the temperatures at which *Fusarium lini* Bolley grows best in pure culture and those at which flax wilt is most destructive.

Sweet potatoes (*Ipomoea batatas* Poir.) are affected by several soil-inhabiting organisms—*Ceratostomella fimbriata* (E. and H.) Ell., the cause of black rot; *Monilochaetes infuscans* Ell. and Hals., the scurf organism; *Fusarium batatatis* Woll., and *F. hyperoxysporum* Woll., the stem-rot organisms; and others. Judging from the amount of infection that occurs in some seasons, there are grounds for believing that the severity of some of these diseases is fundamentally correlated with environmental conditions such as soil moisture and soil temperature.

Poole [6] grew sweet potatoes in pox-infested soils of different water content and found that abundant infection occurred only when it was dry and that no infection occurred in wet soils. Harter and Whitney [7] demonstrated that soil moisture is not a limiting factor in infection by the black-rot organism, *Ceratostomella fimbriata*, inasmuch as infection would occur at any moisture content permitting the growth of the plants. So far as temperature is concerned, however,

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 5
Mar. 1, 1927
Key No. G-563

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it was found that the optimum for the growth of the plants is higher than the maximum at which infection would occur.

Among the diseases of the sweet potato, stem rot is one whose seasonal prevalence and severity seem particularly closely correlated with soil conditions. This disease, which is caused by two species of Fusarium—*F. batatatis* and *F. hyperoxysporum*—appears to be most severe when the weather is very warm and the soil relatively dry during the early part of the growing season.

In order to determine what influence, if any, soil moisture and soil temperature had on infection, an effort was made to control these factors experimentally.

METHODS

TANKS

In these experiments 12 of the so-called "Wisconsin temperature tanks" were used. Each was equipped with an electric control and could be maintained at any temperature desired above 10° C. Inasmuch as most of the investigations were conducted during warm weather, a propeller was installed in each tank to prevent the accumulation of a stratum of warm water at the surface. Notwithstanding this precaution, the temperature varied somewhat from day to day, but any large fluctuations were prevented by the addition of heat or of cold water as necessity required.

The tanks were located in a greenhouse in which there was somewhat less light than would be expected under field conditions. The temperature of the greenhouse ranged from 50° to 85° F. in the daytime and from 60° to 70° at night, depending on the season of the year.

SOIL

The soil employed was a fine sandy loam containing a considerable amount of silt and small gravel and was rather low in organic matter. It was obtained from a low part of the Arlington Experiment Farm at Rosslyn, Va., on which several crops had been grown, and, on the whole, it was well suited for sweet potato culture. Before being used the soil was sifted to remove the gravel and undecayed vegetable matter and was then sterilized by steam for seven hours at 15 pounds pressure. An equal quantity of soil (usually 3,650 gm.) was placed in each can of a given series in all the experiments.

The relation of soil moisture to infection was studied by varying the water content in the soil, the temperature being kept constant. The soil had a water-holding capacity of 25.4 per cent, a moisture equivalent of 9, and a wilting coefficient of 4.9. The soil moisture was calculated in percentage of its water-holding capacity. In the soil-moisture experiments water was added from time to time. It is obvious, however, that such a procedure would of necessity give questionable results, since no method has yet been developed whereby water can be added uniformly to all parts of the soil.

In studying the influence of temperature on infection, the temperatures were varied and the amount of water in the soil kept constant. At frequent intervals, usually once each day, water was added to the cans to restore them to their original weight. A ½-inch glass tube was inserted about 5 inches into the soil, and through it water was delivered to the interior of the can. Again the objection may be

raised that the water was not uniformly distributed throughout the soil. In this case, however, the water relations were not being studied.

The same soil was used in the different series of experiments. At the close of each experiment it was removed from the cans and both the cans and the soil were sterilized by steam for seven hours at 15 pounds pressure, after which the soil was sifted to remove the roots and any other coarse matter that might affect the results. After the soil was thoroughly mixed a weighed portion was added to each can, together with enough water to bring it up to the desired soil-moisture content.

HOST

The Yellow Jersey, one of the sweet potato varieties most susceptible to stem rot, was used in all the experiments. This variety is popular in some of the northern markets and is extensively grown in the States along the northern border of the sweet-potato belt. Disease-free sweet potatoes were bedded in sand containing a small quantity of silt, and when the sprouts reached a height of 6 to 8 inches they were pulled and the roots immersed in a spore suspension of the stem-rot organisms and set out, two or three plants in a can.

MOISTURE

In the experiments to determine the influence of temperature on infection a fairly high moisture content of the soil was maintained, but when the study of the influence of soil moisture on infection was made, the methods were somewhat altered. The quantity of moisture in the soil ranged from very dry to supersaturation. When very dry soil was used the carrying over of a small amount of water on the roots of the plants to the soil might be expected to alter the results materially. Furthermore, spores suspended in water on the roots might conceivably find a more congenial condition for germination and infection. For these reasons the water was allowed to dry off before the plants were set.

PATHOGENE

The organisms employed in these investigations were isolated from sweet potatoes from various parts of the United States, some of which had been carried in cultures for a number of years. No special care was exercised to maintain the organism in what is called "high cultures" and, for the most part, it was not so maintained. In only a few cases were sporodochia present and in no case pionnotes, there being a preponderance of one-celled spores. The cultures were transferred at frequent intervals, usually to stems of *Melilotus alba* Desc. When infection experiments were to be made, the cultures were removed from the tubes and washed in a vessel of water in which the roots were immersed.

TEST OF INFECTION

Investigators familiar with infection experiments of this type will realize the difficulty of determining with certainty when infection has actually taken place. A border line is frequently found where the judgment of the observer must prevail. Typical cases of infection are easy to diagnose, but the nontypical ones which are frequently met under abnormal environmental conditions are sometimes baffling. In the experiments here reported most of the infected plants

could be detected easily. Infection was apparent on most of the plants in about five days. Those which showed unmistakable infection were pulled up, carefully examined, and recorded. At the close of the experiment all the plants remaining in the cans were carefully removed, the roots washed free of soil, and then examined for stem rot. When no external symptoms of disease were apparent the stem and roots were carefully split open and the fibrovascular bundles examined for evidence of blackening. A number of plants were found with bundles blackened from the roots to the stem which still showed no evidence of disease on the foliage and little if any reduction in vigor. In view of these facts it was finally concluded to classify as infected all plants which showed any blackening of the fibrovascular bundles regardless of whether they exhibited any external symptoms.

Fig. 1.—Influence of soil temperature on the infection of sweet potatoes by the stem-rot organisms

RELATION OF SOIL TEMPERATURE TO INFECTION

In all, nine experiments were conducted to determine the relation of temperature to infection, but of these only the last six were employed in the construction of Figure 1. The first three were regarded as merely preliminary, and were planned to determine the optimum soil moisture for growth and to test the efficacy of the method, the virulence of the organisms, and the reliability of the mechanical equipment. While sweet potatoes will grow within a wide range of soil moistures, the best growth was made at about 60 per cent of the water-holding capacity of the soil, and approximately this amount was employed in all except the preliminary experiments. The conclusions as to what constitutes the minimum, optimum, and maximum temperatures for growth are drawn from the combined results of six separate experiments and are shown in Figure 1. It will be seen that a small percentage of plants was infected at a temperature of 12° C. It is interesting to note in this connection that sweet

potatoes will not survive a temperature of 12°, for at this tempera-
ture the plants gradually die. The optimum for infection was
found to be approximately 30° and the maximum nearly 35°, the
optimum for infection being lower than the optimum for the growth
of the plant. As a matter of fact, the optimum temperature for the
growth of sweet potatoes is very near the maximum for infection,
or approximately 35°. The maximum for the growth of sweet
potatoes is certainly above 38° and well above the maximum tem-
perature for infection.

RELATION OF SOIL MOISTURE TO INFECTION

Field observations indicate that stem rot causes much greater loss
during a dry season than during a wet one, particularly if the weather
is dry for a few weeks immediately after planting. Whether this is
due to the greater number of infections during a dry season or to the
fact that diseased plants are more apt to succumb during periods of
drought has not been determined. Inasmuch as no data were avail-
able to explain the influence of soil moisture on infection, a series of
experiments was outlined to establish within reasonable limits the
minimum, optimum, and maximum quantity for infection. Two
methods of procedure were tried. Soil cultures were made up in
steps of 10 from approximately 10 to 100 per cent of its water-holding
capacity. The temperature of the pots was held near 30° C., al-
though there was some fluctuation. The actual water-holding
capacity of the soil was determined at the beginning and at the close
of the experiment. In both methods the sprouts were dipped in a
spore suspension of the stem-rot organisms, planted, and the surface
of the soil covered with about one-half inch of ground cork to reduce
the loss of water from evaporation. By the first method no more
water was to be added to the plants during the entire course of the
experiment. The data obtained by the use of this method were
considered practically valueless, since the time required to obtain
results was so long that the soils of greatest water content had so
dried out that they contained about the same quantity of moisture
as the dry ones.

By the second method enough water was added daily to bring the
pots to their original weight. At the end of the experiments the soil
was removed from the pots and examined. From these examinations
it was noted that the moister soil was either near the surface and out
of reach of the roots or in isolated spots. The soil in the bottom of
the pots was very dry. Figure 2, drawn from the data of two experi-
ments in which the second method was used, shows the results ob-
tained. The water-holding capacity of the soils, which were thor-
oughly mixed before the samples were taken, was found at the close
of the experiment to be approximately the same as at the beginning.
However, the roots were in a much drier soil, so that the results, if
based on the water-holding capacity of the soils, are unreliable.
The results show a high percentage of infection over a wide range of
soil-moisture conditions, while as a matter of fact the roots were
subjected to about the same moisture content—i. e., a fairly dry soil.

Although the data do not give a true picture of the influence of
different quantities of soil moisture on infection, they do show that
infection will take place in a soil containing only enough moisture to
support plant growth. A soil containing moisture equivalent to 14
per cent of its water-holding capacity would not support the growth

of plants, and no infection took place. At 23 per cent of the water-holding capacity of the soil, plant growth was barely supported and 11 per cent of the plants were infected, while at a soil moisture of 28 and 75 per cent with normal plant growth, 94 and 100 per cent infection, respectively, resulted.

Some difficulty was experienced in determining infection in saturated and supersaturated soil because of the fact that the plants rotted below the soil line. Under these conditions some blackening of the fibrovascular bundles in the underground part of the stem resulted, and occasionally the blackening extended into the aerial portions of the stem. Hence, the 45 per cent infection in supersaturated soil, shown in Figure 2, may or may not have been caused by the stem-rot organism, especially since a similar condition occurred in some of the control plants.

METHOD OF INFECTION

In conjunction with the soil-moisture and soil-temperature studies some related problems were investigated, the results of which can be presented appropriately at this point. As a result of field observations it is generally admitted that a large number of stem-rot infections take place in the field. The correctness of these observations has been demonstrated by greenhouse experiments with infested soil. In the study of the relation of temperature to infection the plants were dipped in a spore suspension and then planted in disease-free soil. At the termination of some of these experiments healthy plants were set in the pots from which diseased plants had been removed. Out of a

FIG. 2.—Influence of soil moisture on the infection of sweet potatoes by the stem-rot organisms

total of 34 plants treated in this manner 6 became infected, that is, about 18 per cent. The plants employed in these experiments were wounded at the point where they were pulled from the mother potato, and such wounding no doubt contributed to the ease of infection.

Considerable doubt has existed in the minds of some investigators as to whether wounding is necessary for infection. To obtain information on this point two groups of experiments were conducted in the greenhouse in sterilized soil at a constant soil temperature of 30° C. The excessive loss of water by evaporation was reduced to a minimum by covering the soil in each pot with 35 gm. of ground cork and the ratio of water to soil was kept constant by the daily addition of water. The sweet potatoes (Yellow Jersey) were sound, smooth, and free from disease and would be considered excellent seed. The potatoes were dipped in a spore suspension of the stem-rot organisms

made from several different isolations. The conditions being excellent for sprouting, the first plants appeared above the ground in about five days in both inoculated and control pots.

In the first group of experiments the sprouts were left attached to the potato until the conclusion of the test, when they were all removed and examined for stem rot. In the three experiments of this kind 469 plants were produced on 33 potatoes and a total of 9 diseased plants was observed. The lowest percentage of infection for a single experiment was 0.49 and the highest 5. The second group of experiments was similar in all respects to the first except that at the end of a certain time the plants were pulled from the mother potato and set out in a plant bed. Twenty-seven days after bedding the plants had attained considerable size, but no infection was apparent. Forty-one of these plants were then pulled and set in sterilized sandy loam for further study. Thirteen days later some showed unmistakable symptoms of stem rot. Others became diseased from time to time, until after the plants had been placed in soil 25 days, when the experiment terminated, all but 12, or practically 71 per cent, showed evidence of disease.

The effect of pulling plants from the mother potato on the infection of those remaining may be inferred from the following data: 11 days after the 41 plants referred to above were removed infection was noted in some of those remaining, and 7 days later 26 out of a total of 138 plants were diseased. These results seem to indicate that as long as plants are attached to the mother potato they will not become generally infected, but that as soon as a wound is made by pulling off a sprout, infection will occur.

Free-hand sections of several of the mother potatoes showed that the stem-rot organism which entered at wounds made by pulling off the sprouts had traversed the fibrovascular bundles as far as 3 cm. from the point of entrance. Some of the sprouts in communication with these fibrovascular bundles were infected therefrom.

SUMMARY

Experiments conducted in the greenhouse to determine the relationship between the temperature of the soil and the infection of sweet potatoes by the stem-rot organisms, *Fusarium hyperoxysporum* and *F. batatatis*, showed that the optimum temperature for infection is about 30° C. and the maximum about 35° C. The minimum could only be approximated, since infection occurred at a temperature as low as that at which the plant would grow.

The influence of soil moisture on infection was more difficult to determine. Infection was found; however, to occur over a wide range of soil humidity. At 28 and 75 per cent of the water-holding capacity of the soil 94 and 100 per cent, respectively, of the plants became diseased. Infection also took place in a soil containing only enough moisture to support plant growth.

Healthy plants set in infested soil may become diseased. Wounding is not necessary to infection but increases it. A small percentage of sprouts from healthy potatoes dipped in a spore suspension of the causal organisms became infected. The percentage of infection, however, was greatly increased when the sprouts were pulled, both in those that were pulled and in those that remained.

A TRANSIT DISEASE OF SNAP BEANS CAUSED BY PYTHIUM APHANIDERMATUM[1]

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INTRODUCTION

Species of Rhizoctonia, Sclerotinia, Botrytis, and Rhizopus are frequently associated with, and are unquestionably largely responsible for, a major portion of the losses of snap beans (*Phaseolus vulgaris* Linn.) in transit and on the market. Ramsey [2] has shown that beans may be decayed by *Sclerotinia sclerotiorum* (Lib.) Massee, *S. intermedia* Ramsey, and *S. minor* Jagger, all of which produce a characteristic watery soft rot. The seriousness of the loss in transit caused by Sclerotinia may be inferred from the following quotation from Ramsey's paper:

After selecting at random 42 cars of green beans, which on inspection at market showed Sclerotinia decay, it was found that'the percentage of infection ranged from 5 per cent in some cars to as high as 75 per cent in others, the average for all the cars being 24 per cent.

The symptoms produced by these different fungi vary somewhat, but in certain stages of their development the fungi exhibit characteristics so similar that they can not easily be distinguished. A study of numerous shipments of snap beans from the South after arrival on the northern and eastern markets shows that the symptoms may vary from the partial decay of a few isolated pods to what is referred to by the trade as "nesting," a condition in which as many as a half peck of beans in the center of the pack, or elsewhere within it may be entirely decayed and more or less bound together in a mass of cottony mycelium. When nesting is observed, Sclerotinia or Rhizopus, especially the former, is suspected.

OCCURRENCE OF PYTHIUM APHANIDERMATUM

In the spring of 1925 a bad case of nesting was observed in a container of snap beans shipped from Florida. The beans were overrun with a cottony growth of mycelium, suggesting macroscopically Sclerotinia and isolated as such. A subsequent examination of the culture showed, however, that *Pythium aphanidermatum* (Edson) Fitzpatrick,[3] and not a species of Sclerotinia, had been isolated. Later shipments of beans were examined, and Pythium was frequently observed during the late spring and early summer.

Inasmuch as beans are seldom shipped from the South during the summer months, no more nesting caused by Pythium was found until fall, when it was again observed. Throughout the winter of 1925–26 little or no Pythium was noted. About the middle of April, 1926,

with the coming of warmer weather, Pythium again occurred, the amount of loss increasing with the rise in temperature. These data seem to indicate that the rotting of beans caused by Pythium is to be expected only during the warmer months of the shipping season. This conclusion is tentatively warranted by the fact that the maximum and minimum temperatures for growth of the organism are high, the optimum being about 32° C.[4]

CHARACTER OF DISEASE CAUSED BY PYTHIUM APHANIDERMATUM

The symptoms produced by Pythium are not essentially different from those caused by some other fungi, as, for example, Sclerotinia. A microscopic examination of rotted beans shows that the cells are separated along the line of the middle lamellae. A liquid, probably mostly water, is released in the process of disintegration, thereby reducing the pods to a soft, pulpy mass.

A profuse growth of fine white mycelium, which develops in and about the cells and over the beans, binds the pods so tightly that they are torn into shreds if an attempt is made to separate them.

Pythium causes a very rapid destruction of beans in transit and under artificial conditions. Plate 1, A and B, shows the quantity of decay caused by Pythium in moist chambers at the end of four and five days, respectively, after inoculation.

Inasmuch as the beans are rendered very soft and watery, the term "cottony leak" can appropriately be employed to distinguish this disease from the watery soft rot caused by *Sclerotinia sclerotiorum*, with which it might be confused. Although there is a general similarity between the two diseases, the following gross symptoms will aid in distinguishing them. Sclerotinia frequently produces hard, nearly black sclerotia; Phythium, never. The mycelium of Sclerotinia is coarse, while that of Pythium is fine and more or less fluffy. Sclerotinia has a grayish colored mycelium, while that of Pythium is almost pure white.

INOCULATION EXPERIMENTS

As soon as snap beans were available in the fall of 1925, inoculation experiments were made with *Pythium aphanidermatum* for comparison with *Sclerotinia sclerotiorum*, *Rhizoctonia solani* Kühn, *Botrytis cinerea* Pers., and two species of Rhizopus—*R. tritici* Saito and *R. nigricans* Ehrb. In these experiments Pythium caused a more rapid decay than any of the other organisms. Beans of the same lot were thoroughly washed and held in moist chambers as controls. Some decay developed later and isolations from a number of pods gave, in almost every case, Pythium, showing not only that the organism was present but that it greatly predominated over other fungi. Several sets of inoculations were conducted with *P. aphanidermatum* in moist chambers at room temperature (about 25° C.). A wire support was placed in the moist chamber about 1.5 centimeters from the bottom and the portion above the support was filled with snap beans, which were then washed in running water. A little

A.—Beans in moist chamber four days after inoculation with *Pythium aphanidermatum*
B.—Same beans, 24 hours later

water was left in the bottom of the moist chamber in order to maintain a relatively high humidity. The inoculations were made at the center of the dish by inserting a quantity of mycelium into a wound made by breaking a pod into two parts. The mycelium spread from the point of inoculation in a radial direction to sound pods, eventually reducing them to a pulpy, watery mass. At the end of about five days most of the beans were partially or entirely decayed. (Pl. 1, B.)

Inasmuch as Pythiums are probably present in all types of soil, it was thought advisable to conduct a preliminary experiment to determine the pathogenicity of a number of strains[5] and species isolated from various hosts. Table 1 shows the strain (or species), the host from which it was originally isolated, the source of the culture, and the diameter of the nest formed in moist chambers at the end of five days.

On December 9, 1925, 1 bushel of snap beans of the Hodson wax variety was purchased on the open market in Washington, D. C., and apportioned among twenty 22-centimeter moist chambers. The beans were free of blemishes and were treated as described above. The first records were made two days after the inoculations and thereafter daily for three days.

TABLE 1.—*Species of Pythium, host from which it was isolated, source of culture, daily diameter of the decayed area, and average daily increase in diameter for the period of five days*

Species	Host from which isolated	Source of culture	Diameter of rot in centimeters				
			2 days	3 days	4 days	5 days	Average daily increase
Aphanidermatum --	Bean	Original	S [a]	12 0	15.0	17 0	3.4
Do	do	do	S	8.0	11 0	15 0	3 0
Do	do	Reisolation	S	13.5	19.0	22 0	4.4
Artotrogus	Pea	Drechsler	O [b]	0.0	0.0	0.0	0.0
Complectens	Pelargonium	do	O	0.0	0.0	0.0	0.0
Debaryanum	Pea	do	M [c]	0.0	0.0	0.0	0.0
Do	do	do	S	2.5	4.0	4.5	0.9
Myriotylum	Cucumber	do	O	5.0	13.0	15.0	3.0
Splendens	Pelargonium	do	S	4.0	8.0	9.0	1.8
Ultimum	Bean	Original	M	7.0	11. 5	13.0	2.6
Do	Cabbage	Drechsler	S	5.5	9.0	11.0	2.2
Do	Sweet potato	Original	O	3.0	7. 5	9.0	1.8
5825	Pear	Drechsler	O	0.0	0.0	0.0	0.0
5826	Watermelon	do	O	0.0	0.0	0.0	0 0
5828	do	do	O	0 0	0.0	0.0	0.0
5831	Corn	do	O	0.0	0.0	0.0	0.0
5876	Watermelon	do	O	0.0	0.0	0.0	0 0
Controls (3)			O	0 0	0.0	0.0	0.0

[a] S = Decay is starting but not advanced enough to be measured.
[b] O = No indication of decay or mycelial growth.
[c] M = Mycelium starting to grow but no decay.

Table 1 shows that five species (*Pythium aphanidermatum, P. ultimum* Trow, *P. debaryanum* Hesse, *P. splendens* Braun, and *P. myriotylum* Drechsler, ined.)[6] are parasitic on beans. Of these five only two, *P. aphanidermatum* and *P. ultimum*, were isolated from beans. If the amount of decay produced in a given time (five

[5] "Strain" as here used refers to different isolations of the same organism and does not imply any morphological difference.
[6] This is a new species named by Drechsler, the description of which will be published later by him. It causes a pathological condition in cucumbers very similar to the "leak" caused by *P. aphanidermatum.*

days) is accepted as a criterion, *P. aphanidermatum* would seem to be the most aggressive parasite, followed in order by *P. myriotylum, P. ultimum, P. splendens,* and *P. debaryanum.* It is interesting to note in this connection that *P. debaryanum,* while able to cause decay when inoculated into a wound, was unable to attack sound tissue. On the other hand *P. aphanidermatum, P. ultimun, P. splendens,* and *P. myriotylum,* species penetrated seemingly unwounded cells with apparent ease.

Inoculations were made with three species of Pythium having spiny oogonia (*P. artotrogus* Mont., *P. 5828,* and *P. 5826*), none of which proved to be pathogenic. *P. 5828* has been briefly characterized by Drechsler[7] as having lobular zoosporangia grouped in "a mulberrylike aggregation consisting frequently of more than a score of subglobose communicating elements, from which the contents are delivered through an evacuation tube into a vesicle giving rise to more than a hundred zoospores. The other spiny form [*P. 5826*] associated with decay of watermelons exhibits sporangia that may be regarded as a modification of the subspherical type, consisting generally of a subspherical part together with an adjacent part of one or both hyphal elements between which it is intercalated, the evacuation tube arising from the venterlike part, or from the filamentous part, or very frequently from near the juncture of the two."

Pythium 5876, the third species from watermelon is, according to Drechsler, incompletely known and has not yet been determined. A Pythium identical with *P. 5825* has been isolated frequently from the roots of beans from Florida, but it seems incapable of causing any considerable decay of snap-bean pods. When inoculations were made according to the method described above, some softening of the pods adjacent to the center of inoculation took place, but the results plainly indicated that this species was incapable of causing any considerable damage. This species has never been isolated from decayed beans obtained from the markets, indicating that it is not likely to be of much consequence in transportation.

Little is known at present about the prevalence and distribution of the "leak" of beans. Inasmuch as this organism has been found associated with diseases of plants as widely separated generically as eggplant[8] (*Solanum melongena* var. *esculentum* Nees) and cucumber (*Cucumis sativus* Linn.), it is probably safe to predict that it may be found on beans from widely separated regions.

CONTROL MEASURES

From our present knowledge of the cottony leak of beans, certain recommendations for its control can safely be made. The evidence indicates that the disease originates in the field on beans resting on or partly covered by the soil. If such infected beans are shipped it is probable that some decay will result, the amount depending upon the promptness with which the beans are handled and conveyed to the market and the temperature and humidity to which they are subjected from the time they are gathered until they are marketed.

[7] DRECHSLER, C. THE COTTONY LEAK OF CUCUMBERS CAUSED BY PYTHIUM APHANIDERMATUM. Jour. Agr. Research 30: 1035–1042, illus. 1925.
[8] DRECHSLER, C. THE COTTONY LEAK OF EGGPLANT FRUIT CAUSED BY PYTHIUM APHANIDERMATUM. Phytopathology 16: 47–50, illus. 1926.

As decay is likely to occur in any container holding infected beans, it is advisable to grade the beans carefully and to discard any with blemishes or disease lesions before they are packed. The development of cottony leak is facilitated by high humidity and a fairly high temperature, and for this reason beans should be gathered, if possible, when the vines are dry, and kept in a cool dry place until they are shipped. To allow beans to remain in the hot sun for several hours or more before shipping provides the optimum conditions for the start of a decay which may cause considerable damage during transit. Beans are often wet when they arrive at the markets, showing that they have either been packed wet or that they have "sweat" during transit as a result of insufficient ventilation in the containers, improper ventilation of the car, or both.

In brief, for successful shipment it may be recommended: (1) That the beans be carefully graded and that any showing blemishes and diseased spots be thrown out; (2) that they be picked when the vines are dry; (3) that they be kept in a cool dry place while awaiting shipment; (4) that shipment be made in containers loose enough to permit the maximum amount of ventilation consistent with freedom from the danger of leakage and breakage; and (5) that precooling and refrigeration with adequate ventilation of the car be practiced where the crop is large enough to be shipped in carload lots. If care is taken to handle the crop under the best possible conditions from the time the beans are picked until they reach the consumer, little loss should result.

SUMMARY

A transit disease of beans (*Phaseolus vulgaris* Linn.) caused by *Pythium aphanidermatum* (Edson) Fitzpatrick has been investigated. This organism produces a "nesting" resembling that caused by Sclerotinia, and in general appearance is indistinguishable from the latter except in a few minor details. *P. aphanidermatum* causes a more rapid destruction of beans than Sclerotinia and has a more abundant growth of almost pure white cottony mycelium.

Inoculation experiments were made with a number of other species of Pythium some of which, although not isolated from shipped beans, proved to be parasitic. *Pythium ultimum*, *P. splendens*, and *P. myriotylum* ined. named species were able to produce nesting similar to that caused by *P. aphanidermatum*. One isolation of *P. debaryanum* caused some decay when inoculated into a wound but was unable to attack sound tissue.

None of the Pythiums with spiny oogonia which were tried were found to be parasitic.

Pythium aphanidermatum is very common on beans in transit during the warmer months of the shipping season.

A TECHNIC FOR USE WITH HOMOPTEROUS VECTORS OF PLANT DISEASE, WITH SPECIAL REFERENCE TO THE SUGAR-BEET LEAF HOPPER, EUTETTIX TENELLUS (BAKER) [1]

By Walter Carter

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INTRODUCTION

So far as the writer is aware, no apparatus has yet been reported by means of which the virus causing curly-top of sugar beet can be separated from both the insect vector and the host plant. In the course of studies of *Eutettix tenellus* (Baker) and its relation to curly-top, such an instrument was devised.

METHODS AND APPARATUS

At the beginning of these studies it was found necessary to evolve some method by which the insect could be fed artificially. Pads of thick filter paper soaked with food solution were first used, the insects being kept on them in Petri dishes. This method failed because the pads soon developed molds and could not be kept even moderately sterile. The activity of the insect also made difficult the frequent transfers that were necessary where the Petri-dish method was used. The next trials were with membranes made of collodion on a cheesecloth base, these being sealed over the ends of test tubes containing the food solutions. In a few cases *E. tenellus* was induced to feed through these membranes for periods of several days. As a rule, however, collodion membranes become very hard and this method also was abandoned. Gelatin membranes were found to be too temporary in character and they provided a fertile substratum for fungous growth.

Through a local drug store an animal mesentery was obtained which is sold under the name of "fish-skin." It has been tested quite thoroughly and has thus far proved to be the most practical of any of the materials tried. No. 4 rubber stoppers are drilled out with as large a drill as can be used and a small circle of the membrane fastened over the hole in the large end of the stopper, either with shellac or with a glue made of automobile celluloid dissolved in acetone. Any waterproof adhesive will no doubt suffice. This stopper is then fitted into a short tube containing the food solution. A larger stopper holds the tube in place in a glass cylinder which serves as a cage for the insects. This arrangement is illustrated in Figure 1, A.

For the feeding of a large colony, or where mass action is desired, a small sack may be made of the membrane, which is then filled and hung free in a cage. (Fig. 1, B.) This modification is only of service where the concentration of the food material need not be

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[1] Received for publication Aug. 8, 1926; issued April, 1927.

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kept constant. With the smaller set-up (A) the evaporation sur-
face is very small and little loss is experienced; furthermore, this
equipment has the advantage of making more delicate tests possible.

It has been found that the mortality with this technic is very
high, but whether this is due to the inability of the insect to find
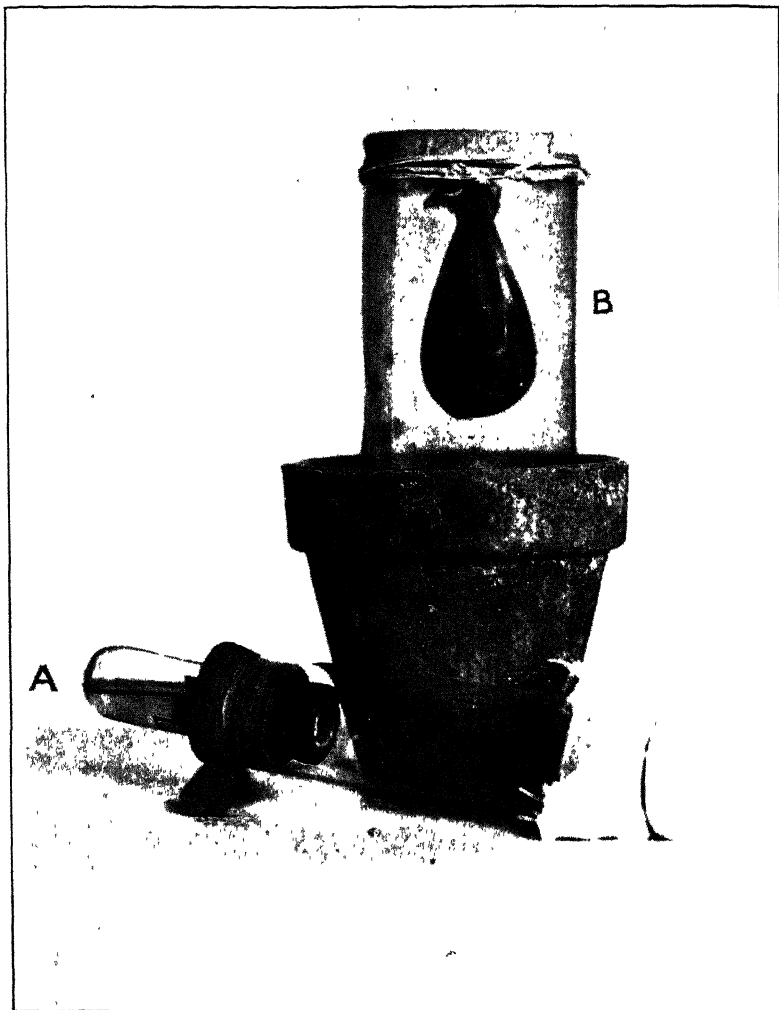
FIG. 1.—Apparatus for feeding *Eutettix tenellus:* A, set-up first used; B, modification of A, employed for feeding insects in large numbers (experience has shown this to be most practical)

the food or to the toxic nature of the food itself is difficult to deter-
mine. That *Eutettix tenellus* feeds through these membranes there
is no doubt since the feeding has been repeatedly observed, and on
one occasion the membrane was peppered with tiny oozings from
the feeding punctures.

ISOLATION OF CURLY-TOP ORGANISM FROM INSECT AND HOST PLANT

A wide range of interesting possibilities is thus opened up, particularly, in this case, with regard to studies on the properties of the virus which the insect carries. For some time the writer did not find it possible by this method to produce the disease from the juice of a diseased beet or from the sap of the wild host plants, but after many attempts the disease has been successfully transmitted from juice expressed from diseased beets to noninfective leaf hoppers and thence to healthy beets by means of this technic. The positive result obtained by the writer was paralleled by similar results obtained by H. H. Severin, of the California Agricultural Experiment Station.

Extracted juice rapidly loses its original character and becomes toxic to the insect, so that it is not safe to feed *Eutettix tenellus* on such juice for more than two or three days at 70° F. Tap water or weak sugar solutions have been the substances on which the writer has been able to sustain the life of the insect for the longest periods. *E. tenellus* fed for two weeks on a dilute cane-sugar solution and one specimen of *Geocoris bullatus* Say was kept alive for a month on tap water.

Nutrition studies with sucking insects should be much simplified by this apparatus, since the quality of the food can be rather exactly controlled. Temperature studies are also facilitated, since the set-up can be used in connection with apparatus for maintaining controlled temperatures—a very difficult matter if it is necessary to feed the insect directly on the plant.

NEW SPECIES AND NEW FORMS OF ICHNEUMONIDAE PARASITIC UPON THE GIPSY-MOTH PARASITE, APANTELES MELANOSCELUS (RATZEBURG)[1]

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INTRODUCTION

One of the most important of the parasites of the gipsy moth introduced into the United States for the control of that insect is *Apanteles melanoscelus* (Ratzeburg). In this country its effectiveness is more or less reduced by the attack of certain native hyperparasites. Six of these hyperparasites are discussed in this paper, four being described as new species.

Hemiteles apantelis, new species

Female.—Length 4 mm. Head minutely granulate, slightly broader than thorax; temples narrower than eyes, rather strongly convex; combined face and clypeus nearly twice as broad as long, with a median elevation extending from below antennae to apex, the clypeus not separated, broadly rounded at apex with narrow reflexed margin; malar space slightly longer than basal width of mandible; cheeks convex; mandibles neither swollen at base nor constricted in middle; antennae as long as head, thorax, and first tergite, first three flagellar joints slender, first and second equal, third slightly shorter, flagellum apically barely thicker than at base. Thorax granulate with only the posterior face of propodeum polished; notauli subobsolete; sternauli fine, extending somewhat more than half the length of pleurum; scutellum strongly convex, not margined; propodeum completely areolated, basal lateral areas large, areola hexagonal with base and sides beyond costulae much shorter than the other sides; stigma nearly three times as long as broad, radius at about the middle, apical abscissa of radius evenly curved, the radial cell shorter on metacarpus than stigma; areolet open, stub of cubitus beyond recurrent parallel with radius; base of second discoidal cell longer than apex of brachial cell; nervulus postfurcal; nervellus inclivous, broken below middle. Abdomen as long as head and thorax, granulate basally, subpolished apically; first tergite flattened, without dorsal carinae, spiracles shortly beyond middle, postpetiole twice as broad as petiole; abdomen subcompressed at apex; ovipositor sheath about twice as long as first segment, slender.

Black, with basal half of antennae, mandibles, and legs beyond coxae dark reddish; wings hyaline with dark veins and stigma and two dusky bands, one below stigma and the other (sometimes wanting) along basal vein, stigma pale at base, tegulae brown.

Male.—Except sexually, essentially like female, but the propodeal carinae are somewhat weaker. the areola nearly equilateral; wings hyaline; legs piceous, with apical joint of front and middle trochanters, their femora at base and apex, their tibiae in front, and extreme base of hind femur and tibia, whitish; antennae black with only scape and pedicel pale beneath; tegulae pale.

Type locality.—Rye, New Hampshire.
Host.—*Apanteles melanoscelus* (Ratzeburg).
Type.—Cat. No. 28058, U.S.N.M.

Described from three females and three males, the two paratype females from Saugus and Boylston, Massachusetts, the males each the progeny of one of the females. The allotype is the progeny of the type.

Gelis apantelis, new species

This species is dimorphic, the male being winged.

In Strickland's key [2] the female runs to *longistylus* (Strickland), but in that species the antennae are nearly as long as the body, very slender filiform, with the first joint of flagellum more than four times as long as thick at apex; the propodeum comprises fully half the total length of the thorax and is barely arched above the level of the mesoscutum; the legs are exceptionally slender; the ovipositor sheath distinctly more than one and one-half times the length of the first segment; and the abdominal color quite different. The male runs nowhere conclusively.

In Brues's key [3] both sexes run to *ottawäensis* (Harrington), but in that species the mesoscutum is very short, the suture separating it from the pronotum is sub-obsolete, and the species belongs to the subgenus Micromeson Strickland. Because of the unusually slender first tergite the true *ottawäensis* runs in Brues's key to *texanus* (Cresson).

Female.—Length 2 to 4 mm. Head twice as broad as thorax and slightly more than twice as broad as thick medially; temples convexly sloping (in the smallest specimen the head is relatively thicker with broader, more strongly convex temples); face nearly twice as broad as long; malar space slightly longer than basal width of mandibles; eye two-thirds as broad as long; diameter of lateral ocellus from one-half to two-thirds the length of ocell-ocular line, which is normally only half as long as postocellar line, but in small specimens relatively longer; mandibles slightly swollen near the base; antennae about two-thirds as long as body, slightly thicker toward apex, flagellum 16 to 19 jointed, first and second joints subequal and about three times as long as thick, others successively shorter, subapical ones fully as broad as thick. Thorax slightly more than twice as long as broad; mesoscutum barely as long as broad, about a half longer than pronotum medially, rather broadly rounded anteriorly; scutellum rather large but very faintly separated, not much shortened; propodeum comprising distinctly less than half the dorsal length of thorax, strongly arched above level of mesoscutum and frequently medially impressed at top, apical carina obsolete to distinct and highly arched medially, pleural carina weak to obsolete; legs rather stout, hind femur hardly five times as long as deep. Abdomen twice as broad as thorax, distinctly longer than head and thorax, with sparse pubescence; first tergite rather slender, barely half as wide apically as long, the sides nearly straight; second tergite barely longer than third, gastrocoeli obsolete; ovipositor slender, sheath a half longer than first tergite.

Head, thorax, first two tergites, and legs ferruginous to fusco-ferruginous; rest of abdomen black; antennae testaceous, black at apex.

Male.—Head barely wider than thorax; eyes large; malar space distinctly shorter than basal width of mandible; temples narrow; diameter of lateral ocellus from twice to several times as long as ocell-ocular line; antennae slender filiform, tapering apically, nearly as long as body, first flagellar joint distinctly longer than second. Thorax normal with very large scutellum, granulate; propodeum subcompletely areolated, the longitudinal carinae between the transverse carinae subobsolete; basal area large, quadrangular, areola hexagonal, costulae far before middle; petiolar area not or but little larger than areola and of nearly the same shape; basal areas granulate, others shining rugulose; legs very slender; wings fully developed; stigma hardly three times as long as broad, radius beyond middle. Abdomen narrow, first tergite less than twice as wide at apex as petiole; tergites 1 and 2 longitudinally striate, third obscurely granulate, others subpolished.

Head and thorax black; abdomen black, with third tergite, apices of first and second, sides of second, and frequently basal middle of fourth, yellow; scape and mandibles yellowish; palpi pale; legs testaceous, trochanters pale, hind tibia and all tarsi fuscous; wings hyaline, venation dark, stigma pale at base.

Type locality.—Melrose Highlands, Massachusetts.
Host.—*Apanteles melanoscelus* (Ratzeburg).
Type.—Cat. No. 28059, U.S.N.M.

Described from a series of 8 females and 13 males, all of the males the progeny of 4 of the females, reared at the Gipsy Moth Parasite Laboratory.

The allotype is the progeny of the type.

[2] STRICKLAND, E. H.   THE PEZOMACHINI OF NORTH AMERICA.   Ann. Ent. Soc. Amer. 5 : 115.   1912.
[3] BRUES, C. T.   DESCRIPTIONS OF NEW ANT-LIKE AND MYRMECOPHILOUS HYMENOPTERA.   Amer. Ent. Soc. Trans. 29 : 120, 121.   1903.

Gelis inutilis, new species.

This species is also dimorphic with winged males.

Very similar to *apantelis* Cushman, but differing constantly in the available material as follows:

Female.—Length 3.5 mm. Ocell-ocular line nearly as long as postocellar line (in specimens of the same size in *apantelis* it is only about half as long as ocell-ocular line); scutellum very short and broad, weakly separated; propodeum not arched above level of mesoscutum, not medially impressed at top; hind tibia nearly as thick near apex as femur; first tergite fully twice as long as wide apically; ovipositor sheath hardly a half longer than first tergite.

Color as in *apantelis*.

Male.—Differs from male of *apantelis* as follows: Eyes not especially large; malar space as long as basal width of mandible; diameter of lateral ocellus not or barely as long as ocell-ocular line; hind tibiae rather conspicuously stout.

Type locality.—Eastern Massachusetts.

Host.—*Apanteles melanoscelus* (Ratzeburg).

Type.—Cat. No. 28060, U.S.N.M.

Described from two females and five males, the males progeny of the females, all reared from the host at the Gipsy Moth Parasite Laboratory.

The allotype is the progeny of the type.

Gelis nocuus, new species.

Another dimorphic species with winged males.

The female runs in Strickland's key [4] to *similis* (Strickland), but is at once distinguishable by its much shorter and more slender legs and antennae and less distinct scutellum. In Brues's key [5] it runs to *micariae* (Howard), in which the antennae are even stouter and shorter, being barely longer than head and thorax, and the clypeus is so short that the labrum is partially exposed.

The male runs in Strickland's key to *flavocinctus* (Ashmead), but differs in its entirely black head and thorax and in the color pattern of the abdomen. In Brues's key it runs to *micariae* (Howard), to which it is extremely similar, but from which it can be distinguished by the fact that the clypeus is hardly twice as broad as long and is obliquely impressed on each side at the apex so that it appears subacute, while in *micariae* it is more than twice as broad as long and apically broadly rounded.

Female.—Length 3.5 mm. Head barely twice as broad as thorax and only slightly more than twice as broad as thick medially; temples convexly receding; eyes very slightly divergent below; face hardly twice as broad as long; malar space slightly longer than basal width of mandible, furrow distinct; mandible slightly swollen basally; antennae somewhat longer than head and thorax, rather stout especially beyond middle, flagellum 18-jointed, basal joint hardly three times as long as thick at apex, subequal to second. Thorax hardly twice as long as thick; mesoscutum with prominent tubercles laterally, scutellum large and very faintly indicated; postscutellum obsolete; propodeum with only the lateral portions of apical carina, sloping strongly from basal third and arched only slightly above level of mesoscutum; prepectal carina convergent above with anterior margin of pleurum; legs stout. Abdomen longer than head and thorax by nearly the length of first tergite; first tergite fully twice as long as broad at apex, much longer than second; second and third equal; ovipositor sheath slightly longer than first tergite.

Head and thorax dark ferruginous; antennae paler at base, fuscous at apex; legs testaceous, hind femur and tibia apically more or less infuscate; first two tergites ferruginous; second more or less piceous; others black; extreme apex brownish.

Male.—Head with temples convexly sloping; diameter of lateral ocellus from as long as to distinctly longer than ocell-ocular line; malar space hardly longer than basal width of mandible; antennae nearly as long as body, slender filiform. Thorax granulate, pronotum laterally and propodeum behind more or less rugulose; notauli obsolete; propodeum with apical carina strong throughout, basal carinae medially and pleural carinae distinct, the carinae more or less obsolete, areolet more or less distinctly defined, rather large hexagonal, petiolar

[4] STRICKLAND, E. H. THE PEZOMACHINI OF NORTH AMERICA. Ann. Ent. Soc. Amer. 5: 116. 1912.
[5] BRUES, C. T. DESCRIPTIONS OF NEW ANT-LIKE AND MYRMECOPHILOUS HYMENOPTERA. Amer. Ent. Soc. Trans. 29: 120. 1903.

area much longer than posterior lateral areas; wings large, stigma nearly half as broad as long, with radius beyond middle; legs slender. Abdomen granulate striate basally, subpolished apically, narrow, postpetiole much longer than broad.

Head and thorax black; mandibles, scape, lower angle of pronotum, and legs red, hind tibiae and tarsi slightly infuscate, flagellum black; wings hyaline, venation dark, stigma pale at base and apex; abdomen flavo-ferruginous, blackish at apex.

Type locality.—Shrewsbury, Massachusetts.
Host.—*Apanteles melanoscelus* (Ratzeburg).
Type.—Cat. No. 28061, U.S.N.M.

Described from 6 females and 9 males all reared from the host at the Gipsy Moth Parasite Laboratory, 3 of the males, including the allotype, the progeny of the type.

Gelis bucculatricis (Ashmead)

Pezolochus bucculatricis Ashmead, 1890, U. S. Nat. Mus. Proc. 12: 420, ♀ ♂;
  Dalla Torre, 1901–02, Cat. Hym., p. 615.
Theroscopus americanus Ashmead, 1896, Amer. Ent. Soc. Trans. 23: 211, ♂.
Hemiteles ashmeadii Dalla Torre, 1901–02, Cat. Hym., p. 643 (not *americanus*
  Cresson).
Pezolochus bucculatricis Strickland, 1912, Ann. Ent. Soc. Amer. 5: 139.
Pezomachus americanus Strickland, op. cit.

Only two of the three specimens of the type series of *bucculatricis*, a female and a male, are left. The female is hereby designated the holotype and the male the allotype.

The unique type of *americanus* is practically identical, except in its larger size, with the allotype of *bucculatricis*. Most of the 27 specimens from *Apanteles melanoscelus* are even larger, and include the hitherto undescribed winged male.

The genotype of Pezolochus Foerster is unknown to the writer. That genus is said to differ from Gelis by its short face. The face in *bucculatricis*, while perhaps somewhat shorter than in some other species of Gelis, is not conspicuously so, and the species is certainly a true Gelis.

Much more characteristic of this species is the carination of the propodeum, which is nearly identical in all three forms. The apical carina is strong and describes nearly an even semicircle between the obsolete pleural carinae; all the other carinae are absent. In the winged male both the apical and pleural carinae are somewhat stronger than in the other two forms.

The unsatisfactory original description of the species makes redescription advisable.

Female.—Length 2 to 4 mm. Head nearly twice as broad as thorax; temples convexly receding; eyes fully four-fifths as broad as long, divergent below; diameter of lateral ocellus hardly half as long as ocell-ocular line; face nearly twice as broad as long; malar space nearly as long as basal width of mandibles, with a distinct malar furrow (in larger specimens the head is relatively shorter from front to back, with correspondingly less strongly convex temples, longer postocellar line, and shorter ocell-occipital line (in the series considered in this study all the gradations in these characters are not present, but this is undoubtedly due to the fact that not all the gradations in size are available); mandibles subangularly swollen near base; antennae about three-fourths as long as body, slender, slightly thickened toward apex, flagellum 17 to 20 jointed, first joint about four times as long as thick, second very nearly as long as first, those beyond gradually shorter, those of apical fourth subequal and of equal length and thickness. Thorax more than twice as long as broad; mesoscutum slightly longer than broad, acutely rounded anteriorly, about twice as long medially as pronotum, slightly impressed in posterior middle; scutellum small but distinct; prepectal suture complete, convergent with anterior margin of mesopleurum; propodeum in profile evenly arched from base to carina, thence flat, higher than mesoscutum; legs rather slender, hind femur about five times as long as deep. Abdomen as long as head and thorax, about half as broad as long and twice as broad as thorax, with very short, sparse pubescence; first tergite nearly or quite two-thirds as broad at apex as long, gradually broadening from base to apex, petiole not or only faintly constricted, least so in smallest specimens; second tergite slightly longer than third, gastrocoeli distinct; ovipositor sheath barely as long as first tergite.

Head and thorax pale ferruginous to fuscous with vertex and sutures slightly darker; mandibles pale; antennae pale testaceous basally, more or less infuscate apically; legs testaceous to fuscous, the coxae, at least below, trochanters, tibiae above and basally, and tarsi always of the lighter color; basal two tergites ferruginous, sometimes slightly infuscated with the apices yellowish, other tergites black, with apices indistinctly reddish, as are sometimes also the apical tergites.

Wingless male.—Of same length as female but more slender, with head distinctly less than twice as broad as thorax; larger scutellum; distinct postscutellum, tegulae, and wing vestiges; longer, more slender, filiform flagellum with first joint much longer than second; eyes larger and less strongly diverging below; diameter of lateral ocellus half to two-thirds as long as ocell-ocular line; propodeum not arched above level of mesoscutum, with medially stronger apical carina and more shining petiolar area; narrower parallel-sided postpetiole; and more slender legs.

Color arranged as in female but generally darker; on abdomen the ferruginous color is frequently replaced by fuscous or black, the apices of the first two tergites rather pale yellow.

Winged male.—Compared with wingless male, differs as follows: Head barely broader than thorax, diameter of lateral ocellus from two-thirds to fully as long as postocellar line; thorax of normal form, with fully developed mesothorax and wings; and propodeum relatively smaller. Wings hyaline with a faint cloud below the stigma and slightly infumate along the basal vein; stigma more than half as broad as long, fuscous with base whitish, radius beyond middle; radial cell shorter on metacarpus than stigma; discocubitus angularly broken at or near middle, ramellus frequently present; second recurrent arcuately inclivous; nervulus postfurcal; nervellus slightly inclivous and broken below middle.

Color same as in wingless male.

Redescribed from type and allotype of *bucculatricis;* type of *americanus·* one female and one wingless male from Ohio; one female reared from an Apanteles cocoon in Riley County, Kansas, by J. B. Norton; and 8 females, 12 wingless males, and 7 winged males all reared at the Gipsy Moth Parasite Laboratory from cocoons of *Apanteles melanoscelus.* Many of the latter lot are progeny of certain of the females.

Gelis urbanus (Brues)

Pezomachus urbanus Brues, 1910, Wis. Nat. Hist. Soc. Bul. 8: 70, ♂.

The writer believes he is correct in referring to this species a series of 21 specimens including, in addition to the wingless male, both the female and the winged male. The last two forms were unknown to the author of the species. They are described below. A brief comparative description of the wingless male is also included.

Female.—Length 3 to 4 mm. Head twice as broad as thorax, about twice as broad as long medially from above; temples convexly receding; face nearly twice as broad as long; malar space fully as long as basal width of mandible, furrow distinct, eye three-fourths as broad as long; diameter of lateral ocellus from distinctly less than to about half as long as ocell-ocular line; width of ocellar triangle distinctly less than length of ocell-occipital line; (variation in the head is of the same nature as that in *bucculatricis*); mandibles not basally swollen; antennae three-fifths as long as body, rather stout, slightly thicker toward apex, flagellum 18-jointed; basal joint hardly four times as long as thick, slightly longer than second, those beyond successively shorter, subapical ones quadrate. Thorax with mesoscutum about as long as broad, acutely rounded anteriorly, less than twice as long as pronotum medially; scutellum faintly defined anteriorly; prepectal carina complete, convergent with anterior margin of mesopleurum; propodeum with apical carina complete, highly arched medially, the propodeum in profile evenly arched from base to carina, slightly higher than mesoscutum, pleural carinae wanting; legs rather stout, hind femur less than five times as long as deep. Abdomen longer than head and thorax by about the length of the first segment, half as broad as long and twice as broad as thorax, pubescence very sparse and short; first tergite nearly twice as long as broad at apex, sides nearly straight; second tergite slightly longer than third, gastrocoeli obsolete; ovipositor sheath subequal in length to first tergite.

Head fusco-ferruginous, black above; antennae ferruginous, black at tips; thorax from pale ferruginous to fusco-ferruginous with sutures darker; legs testaceous to fuscous, paler basally and on upper surface of hind tibia; basal two

tergites ferruginous, sometimes yellowish apically; second more or less infuscate basally, others black, in lighter specimens with apex of second tergite and the apical tergites somewhat reddish.

Wingless male.—This is the form described by Brues. Of same length as female but more slender, with distinct scutellum, postscutellum, tegulae, and vestiges of wings, larger mesoscutum and ocelli, shorter malar space, narrower face, lower propodeum, filiform antennae with basal joint of flagellum distinctly longer than second, more slender legs and abdomen. In color of head and thorax like female but averaging darker, with propodeum distinctly darker than meso-thorax; abdomen black with apices of first two tergites yellowish and of third sometimes reddish.

Winged male.—Head barely broader than thorax; diameter of lateral ocellus from two-thirds to fully as long as ocell-ocular line; thorax and wings fully devel-oped; propodeum nearly straight above, with only the petiolar and apical pleural areas defined, pleural carinae obsolete; wings hyaline immaculate, veins and stigma black, latter pale at base, stigma nearly three times as long as broad, radius at apical third, radial cell shorter on metacarpus than stigma, discocubitus subangular before middle; second recurrent arcuately inclivous, nervulus slightly spostfurcal, nervellus inclivous and broken below middle.

Black, the face and pronotum sometimes stained with reddish and the basa two tergites yellow at apex; antennae and legs as in wingless male.

Redescribed from a series of 7 females, 11 wingless males, and 3 winged males, all reared from cocoons of *Apanteles melanoscelus* at the Gipsy Moth Parasite Laboratory. All the males and one of the females are progeny of the other six females.

DISPERSION OF THE ANGOUMOIS GRAIN MOTH TO WHEAT FIELDS[1]

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INTRODUCTION

In contrast to the behavior of the majority of insect enemies of stored wheat, the Angoumois grain moth (*Sitotroga cerealella* Oliv.) begins its attack upon the crop before it is harvested. Were this not the case the control of the species would be simplified and the moth would be rendered incapable of developing the severe infestations which appear over large areas of the wheat region of the eastern part of the United States, where in some years it is a very destructive pest in harvested wheat. Since the elimination of preharvest infestations would reduce the insect to comparative impotency, it is desirable to examine the character of these infestations and to investigate the factors which make them possible.

The principal questions that arise in a study of preharvest infestation are these: What are the sources from which the parent moths fly to wheat fields? How is infestation in the field distributed with respect to these sources? What is the degree of infestation of wheat in the field? Do the insects pass the winter within kernels that are planted in the fall, and are they able subsequently to infest growing wheat? At what stage in the development of the wheat plant in the spring can the species become established in it? This report presents information in answer to the first three of these questions; the answers to the last two have not yet been satisfactorily determined.

Data obtained in 1924, 1925, and 1926 are here summarized and discussed. These are based on inspections of the conditions on different farms and on the infestations found in samples of wheat heads collected at harvest time in the field. Since it is impossible to take samples through the center of a wheat field before the grain is cut without trampling a considerable quantity of it, samples were taken from shocks, and visits to farms were begun as soon as harvesting operations started. Farms were inspected upon which shocked wheat was present within a few hundred feet of the farm buildings. Each sample consisted of three 6-pound paper bags of wheat heads, the bags being filled about three-quarters full. On each farm a sample, designated as No. 1, was taken from two or three shocks which were located nearest to the farm buildings or to other probable sources of moths. In most cases a second sample was taken at a greater distance from the moth sources; and a third sample was taken from shocks still farther away, usually in line with the shocks from

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which samples No. 1 and No. 2 had been obtained. In choosing the area to be sampled, the desirability of avoiding the parts of a field in the vicinity of possible moth sources on neighboring farms was recognized.

Before the wheat was sampled in the field the owner's experiences with the moth were noted so far as possible, and, with a few exceptions, any grain in storage on the place was examined for infestation. A rough map was made of each farm, showing its location, the location of different kinds of stored grain, and the approximate direction and estimated distance of the sampled shocks from possible sources of moths. The apparent condition of the stored grain with respect to moth infestation and moth parasites was also recorded. Any litter remaining on the sites of mows where wheat had been piled before threshing was examined, and in 1925 and 1926 the presence of loose and baled wheat straw was noted.

In 1924 the inspections were made in west-central Maryland, mainly in Montgomery County. Much of the same area was gone over in 1925, and samples were also taken in southern Maryland (St. Mary's County), in the Shenandoah Valley in Virginia, in southeastern Pennsylvania, and on one farm in Delaware. In 1926 farms were inspected and samples were taken on the Eastern Shore of Maryland, in central and northern Maryland, and in southeastern Pennsylvania. The material collected was kept for observation in an outdoor insectary.

SOURCES FROM WHICH THE MOTHS FLY TO WHEAT FIELDS

It has been known for many years that the moths fly from stored grain, in which they pass the winter in immature stages, and lay eggs upon the maturing wheat in the fields. Duhamel du Monceau and Tillet [2] in 1760 and 1761 made convincing observations on the flight of moths to fields of wheat. According to their records, made during a period of very great abundance of the insect, the moths left the infested granaries in large numbers at dusk, and during the early evening they could be seen on the wheat heads in the fields, mating and depositing their eggs. Similar observations have been published since, notably by King,[3] who studied the insect in 1917 in the vicinity of York, Pa. In the spring of that year he found very little wheat on farms. Infested kernels were observed in small quantities on most farms in such situations as floor cracks, in the litter where wheat mows had been, and in some cases in wheat screenings. No positive evidence was found to show that the insect overwintered in ear corn stored in cribs.

During their harvest-time inspections in 1924 the writers made a special effort to locate farms on which wheat was stored in considerable quantities, but only a few such farms were found. From the observations made, it appeared that corn was by far the most important source of moths in 1924. As shown in Table 1, it was either a known or a suspected source on every farm inspected, with wheat in bins as an additional source in three cases, wheat litter on mow

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[2] Duhamel du Monceau, [H. L], and Tillet [M.]. histoire d'un insecte qui devore les grains de l'angoumois, avec les moyens que l'on peut employer pour le detruire. 314 p , illus. Paris 1762.

[3] King, J. L. the angoumois grain moth. Penn. Dept. Agr , Bur. Plant Indus. Circ. 1, 14 p., illus. 1920.

TABLE 1.—*Dispersion of the Angoumois grain moth to wheat fields, 1924*

Farm No	Date of farm inspection and sampling	Sample No.	Probable sources of moths	Estimated distance of sampled shocks from probable sources of moths	Number of emerged moths	Estimated number of kernels in sample	Estimated infestation
				Feet			*Per cent*
1	July 10	1	Corn	250	27	7,800	0 35
		2	do	600	24	10,100	.24
2	do	1	Corn, wheat	300	73	21,100	.35
		2	do	600	10	18,600	05
		3	do	900	5	18,700	03
3	July 11	1	Corn	50	23	9,300	.25
		2	do	250	1	13,900	.01
4	do	1	do	250	15	13,400	11
5	do	1	do	400	22	14,000	.16
6	do	1	do	400	18	8,800	.20
		2	do	650	16	9,000	.18
7	do	1	do	50	20	12,000	.17
		2	do	200	9	12,300	.07
		3	do	600	0	9,800	.00
8	July 12	1	do	100	21	11,500	18
		2	do	400	6	13,500	.04
		3	do	800	3	10,300	.03
9	do	1	do	300	7	9,400	07
10	do	1	do	300	22	9,300	.24
11	do	1	do	250	11	10,000	.11
		2	do	500	0	10,700	.00
12	July 14	1	do	200	46	11,300	.41
		2	do	400	6	12,600	.05
		3	do	600	5	6,400	.08
13	do	1	Corn, wheat	600	12	12,400	.10
		2	do	700	11	13,100	.08
		3	do	1,000	6	12,500	.05
14	do	1	Corn	30	88	8,200	1.07
		2	do	180	16	11,400	.14
15	do	1	do	150	17	9,200	.18
		2	do	300	0	10,000	.00
16	do	1	do	50	70	10,500	.67
		2	do	250	48	12,400	.39
17	do	1	do	50	95	10,400	.91
18	do	1	do	20	18	11,200	.16
		2	do	120	4	10,500	.04
19	July 15	1	do	30	92	8,100	1.14
		2	do	180	15	9,800	.15
20	do	1	Corn; wheat in litter	20	79	6,800	1 16
		2	do	170	28	10,600	.26
21	do	1	Corn	100	10	10,900	09
		2	do	250	2	14,900	.01
22	do	1	do	300	20	14,800	.14
		2	do	500	3	15,900	.02
23	July 16	1	do	100	180	10,900	1.65
		2	do	300	14	14,600	.10
24	do	a 1	do	50	0	7,500	.00
25	do	1	do	200	2	14,300	.01
26	do	1	do	100	224	10,900	2.06
		2	do	300	58	14,600	.40
27	July 17	1	Corn, barley	300	21	10,700	.20
28	do	1	Corn; wheat in litter	40	24	8,900	.27
		2	do	250	11	8,700	.13
29	do	1	Corn	75	37	14,000	.26
30	do	1	Corn, barley	250	81	14,400	.56
		2	do	450	21	13,400	.16
31	do	1	Corn	50	82	6,300	1.30
32	July 18	1	do	250	20	12,700	.16
33	do	1	do	200	55	15,400	.36
34	July 19	1	do	75	7	11,600	.06
		2	do	375	0	12,700	.00
35	do	1	Corn, wheat	150	15	9,400	.16
			Total		1,876	718,400	
			Average	288	30	11,587	.26

a Bearded wheat; all other samples beardless.

sites in two cases, and barley in two cases.   Unrecorded wheat straw was probably an auxiliary source on some farms.   In 1925 the rôle of corn as a moth reservoir was less marked.

It is probable that the unusual abundance of the moth in the wheat crop of 1923 caused a considerable infestation of the corn in the field. The corn crop was good and the carry-over to 1924 was extensive. Field infestation of corn in the fall has been observed by the writers in Maryland; and it probably occurs, to an extent depending on weather conditions and moth abundance in the wheat crop, throughout the eastern soft red winter wheat area, including southeastern Pennsylvania, Delaware, Maryland, Virginia, western North Carolina, and northeastern Tennessee.

Moths were found in stored grain or litter on 27 of the 35 farms visited in 1924. They were observed to fly from ear corn, when the heaps of ears were disturbed, on 26 of the farms. The corn infestations were light except in the case of farm 30, where the moths were very numerous.

Farms 3, 4, 5, 19, and 26 had wheat in storage, but apparently it was uninfested. On farms 3, 4, and 19 only a bushel or two of wheat was on hand. Some of the farmers had fumigated their wheat with carbon disulphide, although the dosages and methods used were usually not such as would be expected to produce the best results.

Farm 6 had oats in storage, but apparently they were not infested. Although no moths were seen in the corn on farms 4, 9, 10, 24, 25, 27, 28, 29, and 33, the infested condition of the samples taken in fields adjacent to the corn (except on farm 24) indicated that moths had emerged from the corn at some time during the ripening of the wheat. This is to be expected, since a number of records are to be found which show that moth emergence from grain is characterized by surges; the insects emerge in large numbers for a few days and emergence then falls off until another period of abundance begins. King [4] noticed that emergence near York, Pa., in 1917, was greatest between June 5 and 15 and that moths were seldom seen after June 20.

Wheat on several of the farms visited in 1924 had evidently produced moths shortly before the writers' inspection. The owner of 900 bushels of wheat on farm 13, where large numbers of parasites but no moths were found, stated that moths had recently been numerous. The condition of several hundred bushels of wheat on farm 2 indicated that emergence had been plentiful, but at the time of inspection no moths were present. The grain swarmed with hymenopterous parasites, however, and a predacious mite, doubtless *Pediculoides ventricosus* Newport.

On farms 20 and 28 litter, consisting of broken straw, chaff, and scattered kernels of wheat, located in barns or barracks where wheat had been piled in mows for curing, was found infested. A moth was seen on shocked wheat in the field on farms 2, 21, and 22 at a distance of 200, 250, and 300 feet from stored corn which harbored moths.

In 1925 the harvesting of wheat began two or three days earlier than usual, and the inspection of farms was begun earlier than in 1924. (Tables 1 and 2.) As in 1924, the farm reserve of wheat was small, and in most cases it was lightly infested. In fact, infested wheat was found on only 3 of the 43 farms visited, Nos. 8, 18, and 26. On farm 26 hundreds of moths were present in the stored wheat. On farm 34 the wheat had recently been sold and was reported by the owner to have been infested. Stored wheat

was found on 12 farms, Nos. 4, 8, 10, 14, 18, 20, 22, 26, 32, 34, 36, and 37. The wheat on farms 10, 14, and 36 was reported to have been fumigated; that on farm 10 was not examined.

As far as stored corn was concerned, conditions were markedly different from those of the previous year. In 1924 the corn crop on most farms did not have time to mature well, and since moth abundance was at a low ebb in the wheat crop of that year, the ripening

TABLE 2.—*Dispersion of the Angoumois grain moth to wheat fields, 1925*

Farm No.	Date of farm inspection and sampling	Sample No.	Probable sources of moths	Estimated distance of sampled shocks from probable sources of moths	Number of emerged moths	Estimated number of kernels in sample	Estimated infestation
				Feet			Per cent
1	June 20	1	Corn	200	0	11,400	0.00
		2	----do	400	0	11,300	.00
2	---do	1	----do	75	0	10,900	.00
		2	----do	300	0	16,500	.00
3	---do	a 1	----do	200	1	8,800	b .01
		2	----do	600	0	7,100	.00
4	June 22	a 1	Corn, wheat screenings	150	3	7,700	.04
		2	---do	400	0	8,100	.00
		3	----do	500	0	8,300	.00
5	---do	1	Straw	150	0	15,900	.00
		2	----do	400	0	14,100	00
		3	----do	600	0	14,500	.00
6	---do	a 1	None	400	0	8,400	.00
		2	----do	550	0	10,500	.00
7	---do	a 1	Corn, straw	250	0	10,000	.00
		2	----do	400	0	11,900	00
		3	----do	550	0	10,900	.00
8	---do	a 1	Wheat	30	4	10,200	.04
		2	----do	180	2	8,800	02
		3	----do	330	0	8,800	.00
9	June 23	a 1	Corn, straw	50	0	8,900	.00
		2	----do	200	0	11,000	.00
		3	----do	350	0	8,700	.00
10	---do	a 1	----do	50	0	7,900	.00
		2	----do	150	0	10,300	.00
		3	----do	250	0	10,200	.00
11	---do	a 1	Corn	60	0	9,800	.00
		2	----do	260	0	7,700	.00
		3	----do	760	0	8,500	00
12	June 26	1	----do	100	0	12,700	.00
		2	----do	200	0	14,200	.00
		3	----do	350	2	16,500	.01
13	---do	1	----do	150	12	15,300	.08
		2	----do	350	0	14,000	.00
		3	----do	950	0 .	13,500	.00
14	---do	1	Wheat	50	10	16,200	.06
		2	----do	200	6	15,800	.04
		3	----do	450	2	17,800	.01
15	-- do	1	Straw	50	5	14,700	.03
		2	----do	200	0	16,300	.00
		3	----do	400	16	18,700	.09
16	---do	1	Corn, straw	35	21	17,500	.12
		2	----do	135	7	18,800	.04
		3	----do	335	0	20,500	.00
17	June 27	1	Corn	200	11	13,700	.08
		2	----do	350	3	15,300	.02
		3	----do	500	0	15,500	.00
18	---do	1	Wheat	150	2	14,600	.01
		2	----do	300	0	13,700	.00
		3	----do	450	0	15,600	.00
19	---do	1	None	300	1	12,500	.01
		2	----do	450	1	14,900	.01
		3	----do	600	c 0	16,000	.00
20	---do	1	Straw, wheat in litter	100	c 0	14,800	.00
		2	----do	300	0	17,400	.00
		3	----do	400	0	19,100	.00
21	---do	1	Straw	1,000	1	15,700	.01
		2	----do	1,300	0	17,900	C0
		3	----do	1,600	1	16,700	.01

a Bearded wheat in this series of samples.
b Percentages of less than 0.01 are in each case recorded as 0.01.
c On later examination sample was found to have been infested.

TABLE 2 —*Dispersion of the Angoumois grain moth to wheat fields, 1925*—Contd.

Farm No.	Date of farm inspection and sampling	Sample No	Probable sources of moths	Estimated distance of sampled shocks from probable sources of moths	Number of emerged moths	Estimated number of kernels in sample	Estimated infestation
				Feet			Per cent
22	June 27	1	Wheat	300	1	15,200	0 01
		2	do	400	0	14,100	.00
		3	do	500	0	14,600	.00
23	June 29	1	Corn, straw	75	0	16,500	00
		2	do	225	9	14,900	.06
		3	do	375	2	15,900	.01
24	do	1	Straw	109	4	16,700	02
		2	do	200	2	16,800	01
		3	do	300	0	17,000	00
25	do	1	Corn, straw	100	5	16,600	03
		2	do	150	c 0	16,300	.00
		3	do	400	1	15,200	.01
26	June 30	1	Corn, wheat	350	6	14,400	.04
		2	do	500	14	17,400	.08
		3	do	650	5	17,000	.03
27	do	1	Straw	300	8	14,600	05
		2	do	450	c 0	17,500	.00
		3	do	600	c 0	20,100	00
28	do	1	Straw, wheat in litter	30	18	16,900	.11
		2	do	130	0	15,000	00
		3	do	230	3	15,400	02
29	do	1	Corn, straw	1,000	8	14,600	.05
		2	do	1,100	0	17,700	00
		3	do	1,200	3	16,700	.02
30	do	1	Corn; wheat screenings	150	1	15,600	01
		2	do	250	8	14,800	05
		3	do	350	0	11,100	.00
31	July 1	1	Corn	150	6	12,600	.05
		2	do	250	8	16,500	.05
		3	do	350	1	16,900	.01
32	do	1	Straw, corn, wheat	15	4	13,200	.03
		2	do	200	2	16,100	01
		3	do	d 215	1	15,900	.01
33	do	1	Straw	150	8	18,300	.04
		2	do	250	c 0	17,400	.00
		3	do	350	0	18,100	00
34	do	1	Straw, corn, wheat	300	1	15,300	01
		2	do	400	2	17,700	.01
		3	do	500	10	16,800	.06
35	do	1	None	400	2	15,000	01
		2	do	600	0	13,700	00
36	July 2	a 1	Straw, wheat	270	3	10,900	03
		2	do	400	5	10,600	.05
		3	do	500	0	10,200	.00
37	do	a 1	Wheat, straw	100	2	11,200	02
		2	do	200	2	11,800	02
		3	do	e 650	7	12,400	.06
38	do	1	Corn, straw	100	21	11,300	.19
		2	do	250	10	12,800	08
		3	do	400	11	16,500	.07
39	do	1	do	200	29	13,500	.21
		2	do	300	2	16,600	.01
		3	do	400	0	18,000	.00
40	July 3	1	Straw	75	45	15,800	28
		2	do	175	7	17,000	.04
		3	do	275	9	16,000	06
41	do	1	do	75	0	18,900	.00
		2	do	173	4	18,500	.02
		3	do	325	c 0	16,200	00
42	do	e 1	Corn	100	5	10,700	.05
		2	do	175	7	11,300	.06
		3	do	250	1	13,400	01
43	do	1	do	75	0	18,400	.00
		2	do	150	0	17,900	.00
		3	do	225	1	17,600	.01
			Total		425	1,775,900	
			Average	332	3 4	14,321	.02

a Bearded wheat in this series of samples
c On later examination sample was found to have been infested.
d Distance given from a second probable source of moths
e This sample was 150 feet from the premises of a neighbor.

corn was not exposed to much infestation. The small crop and high price combined to produce a small carry-over. Farmers sold or fed so large a part of their corn that the stocks on hand at the time of wheat harvest in 1925 were usually small. In fact a considerable number of farmers were buying corn to supply their current needs.

Of the 43 farms reported upon in Table 2, 18 had no corn in storage. Nine farms (5, 6, 15, 19, 21, 27, 33, 35, and 41) had no home-grown stored grain of any kind. In some cases small quantities were being purchased from time to time. However, all but 3 of these farms (6, 19, and 35) had wheat straw on them, either baled, piled loose, or scattered in barns, barracks, or barnyards. Field infestations occurred on 7 of the 9 farms—15, 19, 21, 27, 33, 35, 41.

On 5 of the 25 farms that were carrying stored corn, the corn was found to be infested. The condition of the corn on farms 7, 38, 39, and 40 was not observed. Where infestation was seen in corn (farms 2, 3, 13, 26, and 30) it was very light. The small quantity of corn on farm 2 was so lightly infested that the samples showed no field infestation. No barley was found in storage, and oats were being held on but 1 farm, No. 36. No moths were seen in the oats. Summing up, moths were observed in stored grain on 7 of the 43 farms visited in 1925; whereas, in 1924, 27 of the 35 farms inspected had shown infestation. A moth was seen on wheat in the field on farm 13, at a distance of 150 feet from infested corn.

In 1924 definitely infested farms were sought, and only those on which stored grain was found were investigated by sampling the near-by shocked wheat. A more general program was followed in 1925, and samples were taken on farms where no grain was found or where straw constituted the only suspected source of moths. Only wheat straw of the 1924 crop was considered capable of producing moths; rotting straw ricks two or three years old were present on some farms, but these were evidently not in a condition to harbor moths.

Straw was found on 22 of the 43 farms. Baled straw under cover of a roof was present on farms 34 and 40; straw piles were found near the barns on Nos. 5, 7, 9, 15, 20, 21, 23, 27, 29, 33, and 37; straw piles were in the fields on Nos. 24, 25, 28, and 32. Litter was found in yards on farms 10, 16, 36, and 41, and under cover in barns and barracks on Nos. 28 and 38. In the absence of other sources, it seems probable that most of the moths which infested wheat before harvest on farms 15, 21, 24, 27, 28, and 33 came from infested kernels that were present in straw. Wheat screenings were found on farms 4 and 30.

In 1926 harvesting of wheat began in central Maryland on June 30, about nine days later than normal. Farm inspections showed an abundant supply of corn on farms from the good crop of 1925. Moths were observed in stored grain or straw on 23 of the 30 farms examined. The corn on these farms was infested without exception, the infestations appearing to be light except on farms 2 and 11. No moths were seen on farms 1, 9, 24, 26, 28, 29, and 30. (Table 3.)

Wheat was found in storage on seven farms—4, 5, 6, 23, 25, 26, and 30. The quantity of stored wheat was generally small, and only that on farms 4 and 5 was seen to be infested.

Barley was present on two of the farms and rye on one, but no moths were observed in these grains.

Straw was found on 18 of the 30 places visited. There was baled straw on six farms, and moths were observed about such straw on farms 7, 13, and 21. Straw litter was infested on farm 21. Loose straw, in more or less well-made piles, was heaped near the barn on 12 farms and inside the barn on 1 farm.

The amount of wheat present in straw after threshing appears to be by no means negligible with respect to the overwintering of the Angoumois grain moth in wheat kernels. The sprouting of wheat kernels present in outdoor straw piles in Montgomery County, Md., during an 11-day rainy period in August, served to indicate the presence of a considerable quantity of grain. Wheat sprouted in all parts of the piles, and in places thick mats of sprouts appeared. Although baling such straw would be expected to prevent the emergence of most of the moths which develop deep within the bales, kernels occur at or near the surface and in the litter that is shaken from bales during handling and when they are cut open for use.

TABLE 3.—*Dispersion of the Angoumois grain moth to wheat fields, 1926*

Farm No	Date of farm inspection and sampling	Sample No	Probable sources of moths	Estimated distance of sampled shocks from probable sources of moths	Number of emerged moths	Estimated number of kernels in sample	Estimated infestation
				Feet			*Per cent*
1	June 24	1	Corn	125	16	10, 600	0. 15
		2	do	225	0	8, 500	00
		3	do	325	0	10, 700	. 30
2	do	1	Corn, straw *a*	50	15	7, 800	. 19
		2	do *a*	200	7	8, 100	. 09
		3	do *a*	400	0	8, 800	00
3	June 25	1	Corn *a*	150	1	8, 400	01
		2	do *a*	250	5	7, 700	07
		3	do *a*	375	15	6, 400	. 23
4	do	1	Corn, straw, wheat *a*	200	5	8, 100	. 06
		2	do *a*	300	5	10, 900	. 05
		3	do *a*	550	0	10, 400	. 00
5	July 2	1	Corn, wheat	250	0	11, 700	. 00
		2	do	375	1	11, 800	. 01
		3	do	575	4	13, 400	. 03
6	do	1	Corn	300	0	12, 600	. 00
		2	do	400	1	13, 900	. 01
		3	do	550	0	13, 800	00
7	do	1	Corn, straw	150	0	14, 200	. 00
		2	do	250	0	14, 800	. 00
		3	do	400	1	12, 400	. 01
8	do	1	do	15	1	13, 700	01
		2	do	165	1	14, 300	. 01
		3	do	315	1	14, 700	. 01
9	July 3	1	do	100	2	12, 800	. 02
		2	do	250	0	14, 300	. 00
		3	do	350	0	14, 700	. 00
10	do	1	do	100	48	14, 600	. 33
		2	do	200	22	15, 100	. 15
		3	do	300	0	14, 300	. 00
11	do	1	Corn	40	50	16, 400	. 30
		2	do	165	13	16, 400	. 08
		3	do	340	0	16, 600	. 00
12	do	1	do	200	0	14, 500	. 00
		2	do	300	10	13, 000	. 08
		3	do	400	1	15, 700	. 01
13	July 7	1	Corn, straw	75	17	9, 500	. 18
		2	do	175	11	11, 600	. 09
		3	do	275	3	13, 500	. 02

a Bearded wheat; all other samples beardless.

TABLE 3.—*Dispersion of the Angoumois grain moth to wheat fields. 1925*—Contd.

Farm No.	Date of farm inspection and sampling	Sample No.	Probable sources of moths	Estimated distance of sampled shocks from probable sources of moths	Number of emerged moths	Estimated number of kernels in sample	Estimated infestation
				Feet			*Per cent*
14	July 7	1	Corn	200	5	12,300	0.04
		2	----do	300	19	13,300	.14
		3	----do	375	1	14,200	.01
15	---do	1	Corn, straw	75	0	13,000	.00
		2	----do	150	0	13,500	.00
		3	----do	250	2	11,900	.02
16	---do	1	Corn, barley	100	2	15,300	.01
		2	----do	175	0	14,800	.00
		3	----do	250	1	16,600	.01
17	---do	1	Corn, straw	100	3	12,700	.02
		2	----do	200	0	15,000	.00
		3	----do	325	36	13,700	.26
18	July 8	1	Corn	125	8	13,600	.06
		2	----do	225	15	12,200	.12
		3	----do	325	2	14,200	.01
19	---do	1	Corn, straw	40	4	13,400	.03
		2	----do	140	1	13,400	.01
		3	----do	240	4	13,000	.03
20	---do	1	Corn	150	0	12,600	.00
		2	----do	250	0	16,600	.00
		3	----do	375	0	16,200	.00
21	---do	1	Corn, straw	50	6	12,900	.05
		2	----do	175	13	14,700	.09
		3	----do	300	1	14,700	.01
22	July 9	1	----do	300	9	13,600	.07
		2	----do	400	0	15,500	.00
		3	----do	500	0	13,900	.00
23	---do	1	----do	100	0	12,500	.00
		2	----do	225	0	12,000	.00
		3	----do	350	0	12,300	.00
24	---do	1	Corn	75	0	11,100	.00
		2	---- do	225	0	12,300	.00
		3	----do	325	0	11,600	.00
25	July 10	1	Corn, straw	500	1	16,500	.01
		2	----do	600	0	16,500	.00
		3	----do	700	0	18,000	.00
26	---do	1	----do	150	42	15,700	.27
		2	----do	250	1	11,900	.01
		3	----do	350	3	14,500	.02
27	July 12	1	----do	100	15	13,300	.11
		2	----do	225	2	14,900	.01
		3	----do	325	2	17,400	.01
28	---do	1	Corn	200	1	14,700	.01
		2	----do	325	0	12,500	.00
		3	----do	425	0	15,500	.00
29	July 13	1	----do	150	1	14,700	.01
		2	----do	250	0	14,100	.00
		3	----do	350	0	17,100	.00
30	---do	1	Corn, straw	40	0	14,100	.00
		2	----do	140	7	11,800	.06
		3	----do	240	16	14,100	.11
Total					479	1,196,600	
Average				254	5.3	13,296	.04

Corn and wheat appear to constitute a cycle of host materials for the moth. In the fall, when the supply of stored corn on farms is at a minimum, moths fly principally from large stocks of infested wheat to the ripening corn; in the spring and early summer, when the supply of stored wheat is at a minimum, the insects fly principally from the generally well-distributed and abundant stocks of corn to the ripening wheat.

DISTRIBUTION OF INFESTATION IN WHEAT FIELDS

The recorded emergence of moths from samples collected in the fields in 1924 shows clearly that the stored grain or litter examined on each farm was the principal source from which the insects flew to the growing wheat. As shown in Table 1, the number of insects present in the shocked wheat in fields adjacent to stored grain decreased as the distance from the source of moths increased.

In 1924 moths emerged from samples collected 800, 900, and 1,000 feet from stored grain. Even though infested planted grain may have been a source of some moths, it is apparent that the distribution of field infestation arising from such moths would not be correlated with the distance to stored grain in farm buildings. In 1924 field infestation was so correlated, with the exception of that on farm No. 12.

The distribution of the infestation in the field was more irregular in 1925 than in the preceding year, according to the records given in Table 2. In 1925 three samples were taken on nearly all farms visited. Shocks at distances of 1,000, 1,200, and 1,600 feet from stored grain or straw were found to be infested. In some cases, as shown in the table, sample 1 was not so heavily infested as were samples at a greater distance from farm buildings, but the average condition was the same as in 1924; that is, the heaviest field infestations occurred in wheat which was nearest to stored grain, litter, or straw. Several samples, originally recorded as free from infestation, were later found to have been infested, as noted in the third footnote to Table 2.

The 1926 sampling showed widespread field infestations to be present at harvest time in the areas inspected. As in the case of the average results for the two preceding years (Table 4), this infestation decreased, in fields near stored grain and straw, with increasing distance from probable sources of moths.

TABLE 4.—*Summary of location, size of samples, and degree of infestation of shocked wheat, 1924, 1925, and 1926*

Year	Sample No.	Average number of kernels in samples	Average distance from probable sources of moths	Average infestation
			Feet	*Per cent*
1924	1	11, 069	173	0. 40
	2	12, 423	360	. 11
	3	11, 540	780	03
1925	1	13, 584	189	04
	2	14, 372	338	. 02
	3	15, 100	185	. 01
1926	1	12, 897	140	. 07
	2	13, 180	250	. 04
	3	13, 810	372	02

Although moths may fly or be blown considerable distances from sources on neighboring farms, the majority of wheat growers in the districts visited provide for most of the infestation of their own wheat fields by maintaining moth sources on their farms. The absence of

such sources during the period in which the wheat is maturing—from blossoming to harvest—is a condition which farmers should try to maintain.

DEGREE OF INFESTATION IN WHEAT FIELDS

Duhamel du Monceau and Tillet[5] found that infestations were heaviest near towns and mills where there was an abundance of infested grain in storage, and that the insects were much less numerous in wheat fields located in clearings in the forest or in the midst of extensive areas of vineyards. A collaborator reported to them that not more than 6 per cent of the kernels were usually infested at harvest time, even in the most heavily infested fields. In one case, however, 330 kernels out of a sample of 3,000 collected at the time of harvest produced moths, an infestation of 11 per cent. Under favorable conditions these small percentages may increase very rapidly. The same collaborator collected a sample of wheat during the threshing, on September 7, 1761, and found the infestation to be over 98 per cent. The wheat had been harvested July 20, at which time the percentage of kernels containing the various stages of the insect was presumably small.

As shown in Tables 1, 2, and 3, the percentage of infestation of all the samples in 1924 was 0.26, of the samples in 1925, 0.02, and of the samples in 1926, 0.04. The average degree of infestation was smaller for 1925 than for 1924 for several reasons. Proportionately more No. 3 samples, which are ordinarily lightly infested, were collected in 1925. There was less stored grain on the farms at the time of the 1925 harvest, and it was more lightly infested. Finally, as previously noted, some of the late-emerging individuals were not included in the emergence records of 1925. When the quantity of material is large there are practical difficulties which prevent the accurate recording of the emergence of all the moths which are present in the samples as eggs, larvae, and pupae at harvest time.

It should be mentioned that there is probably a varying amount of concentration of oviposition on the exposed heads of shocks, and from these heads samples were taken. The concentration would be expected to vary both with the length of time the wheat had been in the shock when the samples were taken (usually from 1 to 10 days), and with the type of shock. Long, narrow, uncapped shocks, such as are common in southeastern Pennsylvania, allow the moths easy access to nearly all of the heads; the very large shocks sampled in southern Maryland were so built as to expose a comparatively small number of the heads in a field.

Eleven of the farms inspected in 1924 were again examined in 1925. The numbers of these farms are paired as follows, the 1924 number being given first: 4 and 23, 5 and 24, 6 and 19, 9 and 21, 10 and 20, 19 and 14, 21 and 25, 22 and 29, 26 and 26, 31 and 30, 33 and 34. Field infestation occurred on these farms in both years, and two of them, 19 and 14 and 26 and 26, were rather heavily infested both years.

[5] DUHAMEL DU MONCEAU [H L], and TILLET [M.]. HISTOIRE D'UN INSECTE QUI DEVORE LES GRAINS DE L'ANGOUMOIS; AVEC LES MOYENS QUE L'ON PEUT EMPLOYER POUR LE DÉTRUIRE. 314 p, illus. Paris, 1762.

Of the farms visited in 1926, nine had been inspected in one or both of the preceding years. Every sampling of shocked wheat on these farms showed that it was infested. The wheat fields near stored grain or straw on farms 18, 19, 22, and 25 were found infested for three years in succession.

Although moths were not observed in grain or straw on farms 1, 9, 26, 28, 29, and 30, the samples showed that the shocked grain was lightly infested on each of these farms. Conversely, on farms 20 and 23, where moths were seen in stored corn, none emerged from the field samples of wheat, although a light preharvest infestation was probably present. Even a more thorough method of sampling than that used by the writers might not reveal the presence of a few insects scattered in a wheat field.

The average harvest-time infestation of wheat in the field was low in fields near probable moth sources in 1926, but the percentage of infestation, 0.04, was twice the average for 1925. However, as shown in Table 4, the 1926 samples were taken somewhat nearer to stored grain and straw than were the samples collected the year before.

The method of arriving at the percentages of infestation was as follows: The wheat in each one-third sample was threshed separately and cleaned with a fan. It was then measured in a graduate and one-tenth of its bulk removed, cleaned of broken and diseased kernels, and counted. Ten times the resulting count gave the number of kernels in the one-third sample. The size of the samples is given in Tables 1, 2, and 3. The percentages of infestation, shown in the last column, were obtained by dividing these figures into the corresponding numbers of emerged moths.

The data referring to the size, distance from moth sources, and percentage of infestation of the samples are summarized in Table 4. The 1924 samples were smaller than those of 1925 and 1926 chiefly because in 1924 an epidemic of wheat scab, *Gibberella saubinetii* (Mont.) Sacc., shriveled from 2 to 30 per cent of the wheat in the samples. This disease appeared to be absent from the 1925 and 1926 material. The average sample in 1924 contained 501 heads of wheat, which averaged 23 good kernels per head; no counts of heads were made in 1925 and 1926.

In the several districts visited in 1925 and 1926 no striking differences in the moth situation were observed.

Although the percentages of infestation in the samples appear small, they indicate the presence of a very considerable moth population in the fields at harvest time, when the number of all stages of the insect per acre are considered. The average infestation in 1924 was 0.26 per cent, yet, as shown below, this means that standing wheat in the vicinity of moth sources was infested to the extent of about 28,500 of the insects per acre.

The harvest-time moth population per acre may be estimated as follows: The number of kernels in 3 one-half pints of wheat from the 1924 samples was counted and found to be, respectively, 5,170, 5,434, and 5,637, an average of 5,413 kernels per half pint. This is at the rate of 21,652 kernels per quart, or 692,864 per bushel. In 1924 the average production of wheat per acre in Maryland was 15.8 bushels.[6] Multiplying this yield by the number of kernels that

[6] UNITED STATES DEPARTMENT OF AGRICULTURE. AGRICULTURAL STATISTICS. BREAD GRAINS. U. S. Dept. Agr. Yearbook 1924 563. 1925.

1 bushel is estimated to contain gives 10,947,000 kernels per acre, 0.26 per cent of which is, in round numbers, 28,500, the average number of insects per acre at harvest time in fields near stored grain. The highest infestation recorded in 1924 was 2.06 per cent, which would indicate the presence of 225,000 moths per acre. Assuming that half of these were females and that each would produce on an average 100 eggs (a conservative estimate), the resulting progeny from the moths in an acre of wheat at harvest time would theoretically number about 11,000,000; equivalent to 100 per cent infestation. The potential importance of preharvest infestation is therefore clear. Given unusually hot weather in June and July and plenty of unthreshed wheat, a light infestation at harvest may result in the presence of enormous numbers of moths a few weeks later. Back and Cotton[7] recorded infestations as high as 90 per cent during 1922 in Montgomery County, Md., where threshing had been much delayed, as compared with an infestation of about 2 per cent at harvest time.

DISCUSSION AND CONCLUSIONS

Several sources of error in the material which forms the basis for this report have been suggested. It is not always possible, in a brief inspection, to determine whether or not a suspected source of moths is infested or has produced the insects during the maturing of the wheat in near-by fields. Especially is this true of corn and straw. The presence of straw piles was not recorded in 1924. It is necessary to collect samples on a succession of days, and the difference in the length of time that the sampled heads have been exposed to the oviposition of the moths reduces somewhat the comparableness of the data. Furthermore, when a large quantity of material is involved, it is practically impossible to obtain an entirely accurate record of the emergence of moths from the sample lots of wheat heads. After making allowance for these irregularities, the writers believe that the information here presented constitutes material from which useful conclusions may be drawn. These conclusions are: (1) The principal moth sources which cause preharvest infestation of wheat in the eastern wheat region are ear corn and wheat in straw, bins, and litter from mows; (2) field infestation of the wheat crop in fields near sources of moths is the rule rather than the exception, and the percentage of infestation decreases as the distance from moth sources increases; (3) the percentage of infestation in the field is low, but an infestation of 0.26 per cent indicates a moth population of about 28,500 per acre (owing to the rapidity of increase of the Angoumois grain moth, these early infestations develop into destructive outbreaks[8] when weather conditions are favorable); (4) if preharvest infestation were eliminated, the severe and widespread damage now caused by the insect would be a thing of the past.

[7] BACK, E A., and COTTON, R T. CONTROL OF INSECT PESTS IN STORED GRAIN. U. S Dept. Agr. Farmers' Bul. 1483, 30 p., illus 1926
[8] The writers have considered the causes of moth outbreaks in the following article SIMMONS, P , and ELLINGTON, G. W. THE CAUSES OF OUTBREAKS OF THE ANGOUMOIS GRAIN MOTH. Jour. Econ. Ent. 18: 309–320, illus. 1925.

TWO HYMENOPTEROUS PARASITES OF AMERICAN JOINTWORMS[1]

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INTRODUCTION

This paper gives an account of the life histories of two important parasites of species of Harmolita. They are hymenopterous parasites of the superfamily Chalcidoidea and have been mentioned in literature, though no detailed accounts of their life histories as parasites of the genus Harmolita have ever been recorded. The observations on which this dissertation is based were made at the United States Entomological Laboratory, Charlottesville, Va., during 1919, 1920, and 1921. The adult parasites used in the experiments recorded herein were obtained from material collected in various localities and kept through the winter in the Charlottesville laboratory under as nearly normal conditions as could be provided.

The methods of rearing followed were, for the most part, similar to those described and illustrated in a previous paper.[2] The parasites here discussed frequently deposited eggs more freely in smaller cages than in those used for certain other parasites. These cages are described in another part of this paper.

EUPELMINUS SALTATOR LINDEMAN[3]

This species (fig. 1) was first described from Russia by K. Lindeman[4] in 1877 under the name *Euryscapus saltator*. It was later placed in the genus Mira Schellenberg, which was regarded as synonymous with Euryscapus Foerster.

In 1918 the late W. R. McConnell,[5] of the division of cereal and forage insects of the Federal Bureau of Entomology, gave an excellent account of the life history of this species as a parasite of the Hessian fly. At the suggestion of A. B. Gahan he used the name *Eupelminus saltator* for the parasite. This was the first published record of this parasite in American literature.

DISTRIBUTION

Lindeman[6] reports that this species from Moscow, Russia, is probably widespread in Europe as a parasite of the Hessian fly, although apparently never abundant. McConnell[7] observed it as a

[1] Received for publication Nov 29, 1926; issued April, 1927. Mr Poos resigned on Mar. 15, 1926
[2] Phillips, W. J., and Poos, F. W LIFE-HISTORY STUDIES OF THREE JOINTWORM PARASITES. Jour. Agr Research 21 405–426, illus. 1921.
[3] Family Encyrtidae, subfamily Eupelminae.
[4] Lindeman, K. DIE PTEROMALINEN DER HESSENFLIEGE (CECIDOMYIA DESTRUCTOR SAY) Soc. Imp. Nat Moscow Bul. (n. s) 1: 190–191, illus. 1887
[5] McConnell, W. R. EUPELMINUS SALTATOR LINDM. AS A PARASITE OF THE HESSIAN FLY. Jour. Econ. Ent. 11. 168–175, illus. 1918.
[6] Lindeman, K. Op cit.
[7] McConnell, W. R. Op. cit.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 5
Mar 1, 1927
Key No K-186

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parasite of the Hessian fly, though not abundant, in 9 localities in Pennsylvania, 2 localities in Maryland, and 2 localities in Virginia. As a parasite of the genus Harmolita the writers have records of its
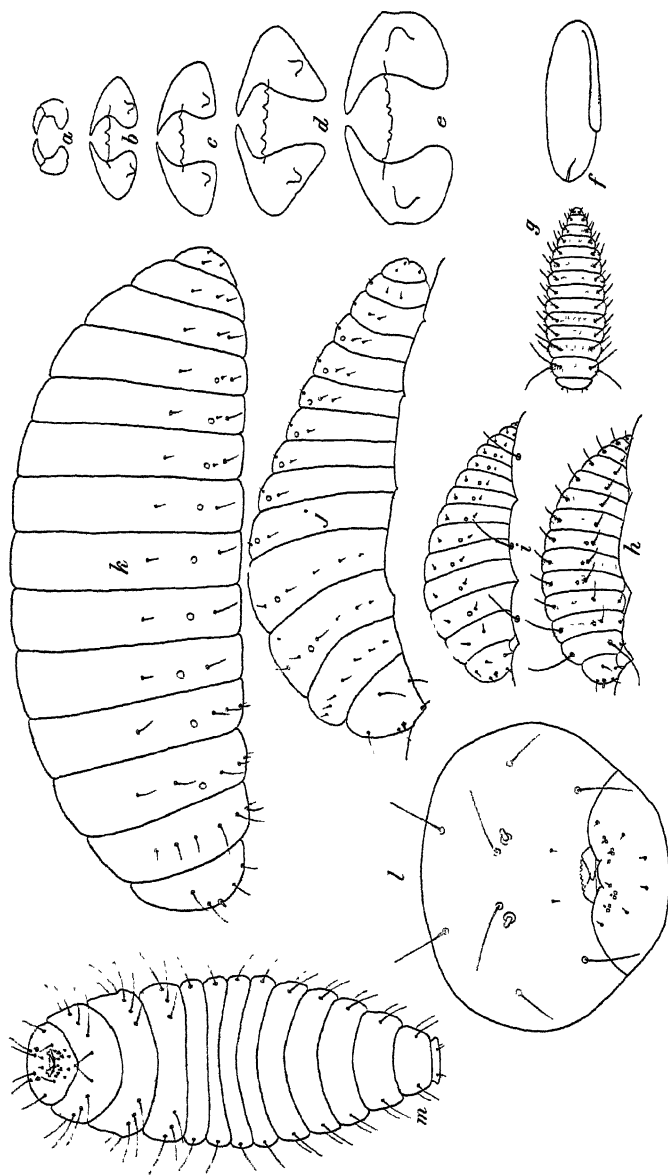
Fig. 1 — _Eupelminus saltator._ _a_, Mandibles of first-instar larva; _b_, same of second-instar larva; _c_, same of third-instar larva; _d_, same of fourth-instar larva, _e_, same of full-grown larva; _f_, egg; _g_, first-instar larva soon after hatching (dorsal view); _h_, first-instar larva just before molting (lateral view); _i_, second-instar larva (lateral view); _j_, third-instar larva (latero-ventral view); _k_, fourth-instar larva (latero-dorsal view); _l_, head of full-grown larva, showing position of sensory hairs and antennae and the sensoria of the mouth parts; _m_, prepupa (ventral view) All greatly enlarged

occurrence from the following States: New York, Ohio, Michigan, Virginia, Pennsylvania, Oregon, Illinois, and North Dakota. This parasite is very widely distributed and probably occurs throughout

the wheat-growing regions of the United States. As a parasite of Harmolita, *Eupelminus saltator* has never been found very abundant except in a few instances when it seemed to be the most important parasite.

HOSTS

The writers have reared *E. saltator* abundantly from several species of Harmolita and are unable to state definitely which species it prefers as a host. It has been reared from the following field collections: From galls of *Harmolita tritici* Fitch, in wheat from New York, Ohio, Michigan, and Virginia; from galls of *H. vaginicola* Doane, in wheat from New York, Pennsylvania, Ohio, Michigan, and Oregon; from galls of *H. hordei* Harris, in barley from New York; from a species of Harmolita in *Agropyron tenerum* Vasey, from North Dakota; from galls of *H. atlantica* Phillips and Emery, in *A. repens* Beauv. from New York; from galls of *H. festucae* Phillips and Emery in *Festuca elatior* L., from New York and Ohio; from *H. maculata* Howard, in cheat (*Bromus secalinus* L.) from New York, Ohio, and Virginia; from *H. dactylicola* Phillips and Emery, in *Dactylis glomerata* L. from New York, Pennsylvania, Ohio, and Michigan; from *H. albomaculata* Ashmead, in timothy from New York, Pennsylvania, Ohio, and Michigan; from *H. elymi* French, in a species of Elymus from Ohio and Michigan; and from galls of Harmolita, in a species of Elymus from Illinois and North Dakota.

Eupelminus saltator deposited eggs freely upon *Harmolita tritici* Fitch, *H. vaginicola* Doane, *H. grandis* form *grandis* Riley, *Eurytoma* spp., *Eupelmus allynii* French, *Ditropinotus aureoviridis* Crawford, *Homoporus chalcidiphagus* Walsh and Riley, and *Phytophaga destructor* Say, when confined in cages. Occasionally this parasite deposited many eggs in empty cells in those cages where there happened to be only a few *H. tritici* larvae and numerous empty cells. Upon one occasion *E. saltator* deposited eggs in the center of green wheat stems which were about ready to head, although no species of Harmolita or Phytophaga were present in this cage. In some cage experiments *E. saltator* deposited eggs in the cells of old galls of *H. tritici* of the previous year in preference to new galls in green stems of wheat when both were present in the same cage. In another instance an egg was deposited in a piece of wheat straw near the point where a puparium of a Hessian fly was located. While the egg was within the straw and near the puparium, the young larva would not have been able to feed upon its intended host.

Eggs of *Eupelminus saltator* which were found deposited upon dead adults of *Harmolita tritici* and upon pupae of *Eupelmus allynii* were transferred with their hosts from the Harmolita cells to glass cell slides. The larvae from these eggs, however, were unable to complete their development upon such hosts. Eggs were often dissected from cells of *H. tritici* where they had been deposited upon larvae of species of Eurytoma. When these eggs were placed in glass cell slides upon their hosts, the very active larvae of Eurytoma frequently crushed the eggs placed upon them, although this was not always the case.

McConnell[8] found *Eupelminus saltator* to be a primary parasite of the Hessian fly, attacking externally both larval and pupal hosts

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[8] McConnell, W. R. EUPELMINUS SALTATOR LINDM. AS A PARASITE OF THE HESSIAN FLY. Jour. Econ. Ent. 11 168–175, illus. 1918.

inside the puparium. He also proved that it may be a secondary parasite of the fly by rearing an individual from a puparium which was found to be filled with cocoons of a species of Polygnotus. *Eupelminus saltator* is ordinarily a primary parasite of Harmolita, but when other parasites are present in abundance it can just as easily maintain itself as a secondary parasite.

No extensive experiments were carried on to determine whether *Eupelminus saltator* preferred Harmolita to the Hessian fly, but in a few instances several adults in cages deposited eggs in cells of *Harmolita tritici* in preference to puparia of the Hessian fly. Upon one occasion a female was observed to oviposit in cells of *H. tritici*, and upon hatching the young larvae were reared upon their hosts in glass cell slides; when adults emerged from these cell slides they were induced to deposit eggs within the puparia of the Hessian fly; those eggs were then removed to glass cell slides, allowed to hatch, and reared to adults upon the pupae of the Hessian fly. These progeny were in turn bred back upon *H. tritici* by the same method. The adults used in these experiments were saved for comparison and were found to be identical in external appearance. These experiments proved that *E. saltator* is entirely able to maintain itself upon either Harmolita or the Hessian fly, which characteristic adds very much to the value of this parasite as an enemy of the two most important wheat pests in the United States.

Eupelminus saltator has been found to have a wider range of hosts than any other parasite of Harmolita, with the possible exception of *Eupelmus allynii*. This wide range of hosts, consisting of various species of Harmolita and their parasites, and the Hessian fly and its parasites, would seem to make this species a particularly desirable and valuable parasite. *E. saltator* will probably increase and aid greatly in reducing the numbers of either of these hosts when they become very abundant.

EGG

The eggs (fig. 1, *f*) are well described by McConnell[9] as follows:

They are white in color. The chorion is thin and elastic, with a smooth and shining surface. They are ellipsoidal in form with a large pedicel at the cephalic pole and a slender flagellum at the caudal pole * * *. The pedicel is about half as long as the egg and is usually folded back along the side of the egg, its tip being recurved forward, but it may be bent and twisted in various ways. The flagellum at the posterior pole is about half as long as the pedicel and usually lies against the posterior surface of the egg.

The average size of three eggs was found to be 0.3966 mm. in length and 0.1508 mm. in greatest width. The period of incubation for 40 eggs ranged from 1.5 to 4 days and averaged 2.3 days. In August, 1921, when weather conditions seemed normal, the incubation period of 13 eggs averaged 2.1 days. Observations on the process of hatching showed that occasionally the mandibles were thrust out with a vicious stroke, as though trying to cut the chorion. The chorion is ruptured at the base of the pedicel, at which point the larva wriggles out of the eggshell.

The eggs are always deposited on the outside of the host. Some seem to cling lightly to the host and others are frequently fastened to the wall of the Harmolita cell by means of a delicate, fibrous,

[9] MCCONNELL, W. R. Op. cit.

netlike structure which is apparently woven from fine white threads. This covering is similar to that of *Eupelmus allynii*, which was described and illustrated by the writers in a previous paper.[10] Not uncommonly four or five eggs, and sometimes more, have been found in one Harmolita cell. In such cases the covered eggs always had a separate, individual covering, although these coverings were usually attached to each other. No cases were observed where several eggs were found under a single cover. The eggs were held securely in place as the coverings were usually fastened down rather firmly all around the edges.

Numerous infertile eggs were found in the breeding cages, a large percentage of which had the weblike covering woven over them. Such conditions may have been due to the fact that the adults were exposed to unfavorable environment, or that they did not receive the proper food. Only those eggs which presented a well-rounded normal appearance and were apparently viable were used in the experiments or removed to glass-cell slides where they could be under close observation.

In describing the larvae all the sensory hairs for the head but only those on one-half of the body were recorded. However, the sensory hairs on only one side of the head are figured except where a full front view is given. This should be kept in mind when reading the descriptions. All drawings were made with a camera lucida from living specimens and consequently all could not be drawn from exactly the same angle. While an attempt was made in every case to get a full lateral view, it was impossible, in a few instances, to do so.

LARVA

The larva of *Eupelminus saltator* falls in Parker's[11] Group VI, but differs slightly from his description of a species of Eupelmus. There are only four pairs of large sensory hairs on the head instead of five; all of the abdominal segments have on each side two large sensory hairs, without exception, whereas Parker mentions only one for the sixth segment and none for the tenth. ·

FIRST INSTAR

The shape (fig. 1, *g, h*) is typical for Parker's[12] Group VI. The color is the translucent whitish; the segmentation is very distinct; the head is brownish and heavily chitinized; the head and body bear numerous sensory hairs of varying length.

In outline the head is elbow-shaped, and reminds one somewhat of the profile of the beak of a vulture; it is approximately as wide as thick but distinctly longer than wide; and is brownish in color and highly chitinized. There are two antennae, approximately three times as long as broad, with only one segment. The head bears eight rather prominent sensory hairs arranged as follows: A pair just above and just inside the antennae, a pair just above and a pair just back of the mandibles; and one sensory hair near the center of each lateral face. The hairs on one side of the head only are illustrated

[10] PHILLIPS, W. J., and POOS, F. W. LIFE-HISTORY STUDIES OF THREE JOINTWORM PARASITES Jour. Agr. Research 21: 405–426, illus 1921
[11] PARKER, H. L. RECHERCHES SUR LES FORMES POST-EMBRYONNAIRES DES CHALCIDIES Ann Soc. Ent France 93: 261–379, illus. 1924. (Thèse Univ. Paris)
[12] PARKER, H. L. Op. cit.

(fig. 1, *h*).   The mouth is situated on the ventral side at the apex of
the head and is surrounded by a chitinized ring; the mandibles are
heavily chitinized, rather long and slender, somewhat sickle-shaped,
and dark brown.   The sensory organs on the labrum and labium are
too obscure to place with certainty.

The body is composed of 13 segments, the greatest diameter of the
larva being at about the first or second abdominal segment, from
which point the body tapers gradually, being very pointed posteriorly.
Each thoracic segment bears on each side three sensory hairs; the
dorsal and lateral ones of the first segment are the longest and coarsest
of any of the body setae; the dorsal setae of the second segment are
somewhat smaller, while the remaining body setae are about of a
uniform size, with the exception of the ventral setae, which are
smaller.   Sensory hairs are arranged as follows: A lateral row the
full length of the body just below the spiracles, a tergal row likewise
the full length of the body, and a sternal row in the thoracic region
only.   Around each segment is a rather distinct band of spicules.
There are four pairs of open spiracles, one on the second thoracic
and one on each of the first, second, and third abdominal segments.

In the newly hatched larva the head is about as broad as the abdom-
inal segments, but just before molting it is very noticeably narrower.

The average length of 3 larvae was 0.5018 mm., the average
greatest width 0.1605 mm.

SECOND INSTAR

After the first molt the larva has a very different appearance.   It is
perhaps more delicate in this stage (fig. 1, *i*) than in any other.   The
setae are very inconspicuous.

The head is somewhat intermediate in shape between that of the
first-instar larva and the full-grown larva; it is very feebly chitinized,
whitish; and it bears 10 small sensory hairs arranged as follows: A
pair just above and a pair just back of the mandibles; a pair between
and just above the antennae; a pair submedian on the dorsal posterior
part of the epicranium, and one hair near the center of each lateral
face.   The antennae are smaller than in the previous instar, about
twice as long as broad; the mandibles are triangular in outline and
feebly chitinized; the sensory organs of the labium are very incon-
spicuous and no attempt was made to place them.   The labrum bears
several heavily chitinized lobes.   The setae in this instar are much
less conspicuous than in the first instar.   The thoracic and abdominal
segments bear on each side two sensory hairs, arranged as indicated
in Figure 1, *i*.   There are nine pairs of open spiracles in this instar.

The average length of five larvae was 1.078 mm., and the average
width was 0.3325 mm.

THIRD INSTAR

Color and general shape (fig. 1, *j*) about the same as in the previous
instar.   The setae are more prominent on the head in this instar
but approximately the same on the body.

The head is shaped very much as in the full-grown larva and is
more strongly chitinized than in the second instar; there are 10
rather prominent setae on the head, arranged as in the second instar;
the antennae are about twice as long as broad; the mandibles are tri-

angular in outline and rather heavily chitinized. No attempt was made to place the sensory organs of the labium; the labrum bears several heavily chitinized denticles (fig. 1, *c*). Setae on the body are slightly more conspicuous than in the preceding instar and more numerous, arranged on each side of the body as follows: Seven setae on the first thoracic segment, 6 on the second, and 5 on the third; 2 setae on each of the first, second, third, and fourth abdominal segments; 3 setae on each of the fifth, sixth, seventh, eighth, and ninth abdominal segments; the tenth abdominal segment bears two setae on the upper lobe and one on the lower. There are nine pairs of open spiracles in this instar.

Three larvae averaged 1.47 mm. in length and 0.49 mm. in greatest width.

FOURTH INSTAR

The color and general shape (fig. 1, *k*) are quite similar to the color and general shape of the previous instar. The setae throughout are more conspicuous than in the previous instar.

The head is shaped as in the full-grown larva and rather strongly chitinized; there are eight rather prominent setae and two smaller setae on the head arranged as follows: A pair of small setae just above the labrum; a pair of prominent setae between and slightly above the level of the antennae; a pair submedian on the dorsal posterior part of the epicranium; one near the center of each lateral face and slightly above the plane of the antennae; one on each side in about the same plane as the mandibles and laterad of them. The antennae are about twice as long as broad; the mandibles are triangular in outline and rather heavily chitinized. No attempt was made to place the inconspicuous sensoria on the labium; the labrum bears four or five heavily chitinized denticles (fig. 1, *d*). Setae on the body are more conspicuous than in the third instar, of equal number and in the same relative position. The illustration (fig. 1, *k*) shows the latero-dorsal aspect of the larva; some of the body setae do not show very well, and only four of those on the head could be shown. There are nine pairs of open spiracles.

Three larvae of the fourth instar averaged 1.75 mm. in length and 0.5833 mm. in greatest width.

FULL-GROWN LARVA

This is cylindrical, tapering toward each extremity (fig. 2). There are 13 distinct segments in addition to the head; the head is almost hemispherical; the larva is translucent-whitish except in the mandibular region where it is brownish. The head is narrower than the body and the mouth parts point downwards. The antennae are about twice as long as broad, unsegmented. There are 8 prominent and 2 very small sensory hairs or setae on the head arranged as follows: 2 small ones just above the labrum; 2 prominent sensory hairs between and slightly above the level of the antennae; 2 submedian on the dorsal posterior part of the epicranium; 1 near the center of each lateral face and slightly above the plane of the antennae; 1 lateral to each mandible and about in the same plane. The labrum is convex, heavily chitinized and usually bears seven prominent denticles. The ventral mouth parts form a fused region which anteriorly is tri-

lobed, corresponding to the component median labial, and the two lateral maxillary regions. This fused region bears 20 sensory hairs and organs arranged as follows: Two groups of sensory organs with 3 in each group in each maxillary region, 4 of which organs are ring-shaped, and 2 are very small sensory hairs; in addition there are 2 more prominent sensory hairs laterally of these groups in each maxillary region, and 4 sensory hairs on the median lobe or labial region (fig. 1, *l*). The mandibles are heavily chitinized, reddish brown, and triangular in outline. The first thoracic segment bears on each side 7 long hairs, the second 6, and the third 5; the first, second, third, and fourth abdominal segments each bear 2 hairs; the fifth, sixth, seventh, eighth, ninth, and tenth abdominal segments, each 3 hairs. These hairs are arranged as follows: A tergal row and a spiracular or lateral row the full length of the body and an additional lateral row on the fifth, sixth, seventh, eighth, and ninth abdominal segments, just below the lateral row; the tenth segment has on each side 2 setae on the upper lobe and 1 on the lower. On the thorax there are, in addition to the lateral and tergal rows, 3 ventral or sternal hairs to each segment, 2 tergal hairs on the first thoracic and 1 on the second segment. There are 9 open spiracles. Usually
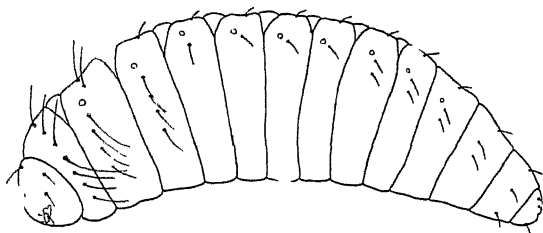
Fig. 2.—Full-grown larva of *Eupelminus saltator*

there are about 8 dorsal folds between the segments, beginning at the interval between the first and second thoracic segments.

Three full-grown larvae averaged 2.601 mm. in length and 0.863 mm. in greatest width.

The manner of feeding and locomotion is similar to that of other chalcidoid parasites of the genus Harmolita. The first larval instar often feeds with only its head touching its host. Larval develop-ment of 106 individuals observed in glass cell slides required from 5 to 19 days and averaged 9 days. The period of time individuals remained as mature larvae before pupating varied from 2 to 28 days, and the average for 82 individuals observed was 6.02 days. Many larvae became full grown in glass cell slides but died before pupating. Some of these individuals remained in apparently good condition for a period of over 18 months; after this time they began to contract, lost their normal appearance, and at the end of about 5 more months, when they were about one-third their normal size, died. The larvae of this species are very active and feed without being easily disturbed. They are cannibalistic; although as many as 10 eggs have been found in one Harmolita cell, in cage experiments, never more than one individual completed its development upon a single host larva, which always seemed to provide sufficient food for the parasite to develop fully. The size of the adult parasite was observed to vary

proportionately with the amount and condition of the food supply. In cage experiments, undersized adults could not be induced to oviposit.

<center>PREPUPA</center>

The prepupal or final developmental period of the full-grown larva (fig. 1, *m*) of 47 individuals of this species ranged from 1 to 3 days, the average being 1.4 days. No excrement is voided by the larva until the prepupal stage is entered upon, when a considerable amount is voided, and the prepupa becomes pure white in color and contracts both in length and width. The third thoracic and the first, second,
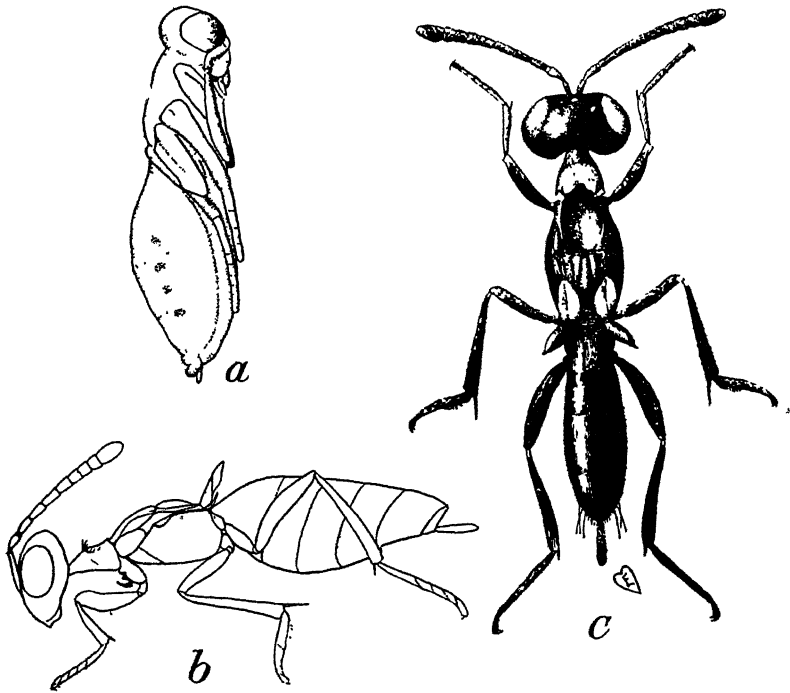
Fig 3.—*E ipelminus saltator* *a*, Lateral view of pupa; *b*, dorsal, and *c*, lateral views of adult. (McConnell)

and third abdominal segments become much shorter. The arrangement of the setae remains as described in the earlier development of the full-grown larva. Actual measurement of two prepupae gave an average length of 2.10 mm. and the greatest width averaged 0.805 mm.

<center>PUPA</center>

When first formed the pupa (fig. 3, *a*) is pure white but gradually turns to a light or reddish brown, which approaches the color of the adult. The pupa is slightly concave ventrally; the head is slightly wider than the thorax, the average greatest width of 7 individuals being 0.71 mm. The average length of these same 7 individuals was 2.57 mm. The duration of the pupal period of 86 individuals observed varied from 7 to 13 days and averaged 9.28 days.

ADULT

No males have been found to occur in this species. The females pass the winter as full-grown larvae in the cells of the Harmolita. In Virginia adults of the first generation begin to emerge in May. The adults of *Eupelminus saltator* (fig. 3, *b*, *c*) are easily distinguished from all other known parasites of Harmolita because they do not possess functional wings. They are unable to fly but are able to leap rapidly many times in succession. In most instances they were observed to walk and jump toward the light. In cages the adults were fed a weak sugar solution, which added greatly to their length of life. The adults are very active, and, in cages, travel up and down the straws, stroking them with the antennae, which are bent forward and downward and kept in constant vibration, as though searching for a suitable place to oviposit. The manner of oviposition is the same as that described for *Eupelmus allynii* in a previous paper [13] and the process usually requires several minutes. Sometimes oviposition was much more readily obtained in shell vial cages 30 by 100 mm. in size containing galls of *H. tritici* than in larger cages described in a previous paper.[14] When *H. grandis* form *grandis* or *H. maculata* were used as hosts, oviposition was often more readily obtained by using a 2 by 12 inch glass cylinder cage than by using larger cages of this type.

Longevity experiments in 1920 showed that eight individuals lived from 16 to 38 days, or an average of 26 days, when kept under the most favorable conditions.

The preoviposition period of eight adults of *Eupelminus saltator* ranged from 3 to 19 days and averaged 7.37 days. When hosts are abundant the average preoviposition period in the field is probably not over 3 to 5 days. Of the eight females referred to above, the maximum number of eggs deposited by a single individual was 26, while one female deposited only 3 eggs; the average for the eight females was 10.7 eggs. As many as 10 eggs were obtained from one female in one day. In 1921 one individual under observation lived for a period of 51 days and deposited 30 eggs; another lived 37 days and deposited only 1 egg; while still another lived 27 days and did not deposit any eggs.

During 1919 six generations of *Eupelminus saltator* were reared in the laboratory, from May to October. The period of time required to develop from the egg to the adult during this breeding season varied from 18 to 43 days. The average for eighty-two individuals under observation was 23.5 days. Low temperatures during the spring and fall seemed to retard development considerably, as might be expected. Furthermore some full-grown larvae remained in the cell slides until the following spring before they changed to adults; this, too, in spite of the fact that apparently every condition favorable for development had been met, since other larvae of the same generation and under the same conditions developed normally. Such retarded larvae would wriggle vigorously when touched and seemed normal in every way except for the period of estivation that seemed necessary for them.

[13] PHILLIPS, W J , and POOS, F. W. LIFE-HISTORY STUDIES OF THREE JOINTWORM PARASITES. Jour. Agr. Research 21: 405–426, illus 1921.
[14] PHILLIPS, W. J., and POOS, F. W. Op. cit.

ERIDONTOMERUS ISOSOMATIS [15]

Stictonotus isosomatis Riley, U. S. Dept. Agr., Rpt. Ent. 1881–2, p. 186.
Stictonotus isosomatis Howard, U. S. Dept. Agr., Bur. Ent. Bul. 5 (old series), 1885, p. 45.
Merisus isosomatis Cresson, Syn. Hym. Amer. North of Mexico, 1887, p. 242.
Semiotellus isosomatis Dalla Torre, Cat. Hym., v. 5, 1898, p. 211.
Eridontomerus primus Crawford, Journ. N. Y. Ent. Soc., v. 15, 1907, p. 179.
Merisus isosomatis Viereck's Hym. Conn., Bul. 22, Conn. Sta. Geol. and Nat. Hist. Surv., v. 3, 1916, p. 478 (description).

This species was first described, both male and female (fig. 4), in 1881 by C. V. Riley as *Stictonotus isosomatis* from material received from J. K. P. Wallace, of Andersonville, Tenn.   A. B. Gahan[16] in 1920 published a paper giving the complete synonymy of the species as it appears above.

DISTRIBUTION

This parasite is very widely distributed and probably occurs throughout all the wheat-growing regions of the United States.   As a parasite of the genus Harmolita the writers have records of it from the following States: Massachusetts, New York, Pennsylvania, Ohio, Michigan, Indiana, Illinois, Virginia, Kentucky, and Arizona.

HOSTS

In the field this species has been found most abundantly as a parasite of *Harmolita maculata* Howard, and has been reared from collections of cheat (*Bromus secalinus* L.) from Massachusetts, New

FIG. 4.—Adult female of *Eridontomerus isosomatis*

York, Pennsylvania, Ohio, Michigan, Indiana, Illinois, Virginia, and Kentucky.   The writers have records of rearings from the following field collections: From galls of *H. festucae* Phillips and Emery in *Festuca elatior* L. from New York, Ohio, and Virginia; from *H. grandis* form *grandis* Riley, in wheat from Virginia; from galls of *H. vaginicola* Doane, in wheat from Michigan; from collections of wheat from Arizona; from galls of *H. atlantica* Phillips and Emery, in *Agropyron repens* Beauv. from New York; from a species of Harmolita in an Agropyron from Michigan; from *H. websteri* Howard in rye from Pennsylvania and Virginia; from *H. dactylicola* Phillips and Emery, in *Dactylis glomerata* L. from New York, Pennsylvania, Ohio, and Michigan; from galls of *H. secalis* Fitch, in rye from Virginia; from *H. albomaculata* Ashmead, in timothy from New York, Pennsylvania, Ohio, Michigan, Indiana, and Virginia; from species of Harmolita in species of Elymus from Virginia.

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[15] Family Callimomidae, subfamily Monodontomerinae.
[16] GAHAN, A. B.   ON THE IDENTITY OF SEVERAL SPECIES OF CHALCIDOIDEA (HYMENOPTERA).   Ent. Soc Wash. Proc. 22: 235–236.   1920.

In cages, the females of *Eridontomerus isosomatis* showed no host preference between *Harmolita maculata* and *H. grandis* form *grandis*. The larva of *H. tritici* Fitch was successfully used as a host for this parasite in glass cell slides. *E. isosomatis* will probably parasitize any of the various species of Harmolita, though it seems to prefer the center-of-stems forms. It has been observed to parasitize individuals of its own species, and though it is usually a primary parasite it undoubtedly becomes secondary when the Harmolita are heavily parasitized.

EGG

The egg (fig. 5, *f*) is grayish white in color and opaque. It is elongate, kidney-shaped; there is a nipplelike process at each pole; the entire surface is covered uniformly with spicules with the exception of the nipplelike process at the posterior pole. The average length of six eggs was 0.5026 mm. and the greatest width was 0.1179 mm. The eggs are deposited in the Harmolita cells external to the host larva, either directly upon the host or in close proximity to it.

LARVA

First Instar

This larva (fig. 5, *g*, *h*) belongs to Parker's[17] Group VI. It is cylindrical in shape, tapering slightly towards each extremity; translucent-whitish; segmentations very distinct; head brownish; the head and body are covered with numerous sensory hairs; the sensory hairs on anterior part of body are much more prominent.

Head (fig. 5, *h*) viewed laterally is somewhat elbow-shaped, about as wide as thick, but longer than wide; brownish in color, heavily chitinized; it bears two antennae, apparently twice as long as broad and with only 1 segment; the head also bears three pairs of fairly prominent setae or sensory hairs arranged as follows: A pair just above the labrum, a pair just above and between the antennae, and one on each lateral face. The mouth is situated on the ventral side at the apex of the somewhat elbow-shaped head; the mandibles are sickle-shaped, heavily chitinized, and dark brown (fig. 5, *a*).

The body is composed of 13 segments about equal in length; it tapers only slightly anteriorly, more posteriorly. Each thoracic segment bears on each side 3 very prominent setae, and each abdominal segment bears 2 setae on each side; the setae in the thoracic region are long, coarse hairs, and those on the remaining body segments gradually diminish in size, until those of the last two segments are almost invisible. The setae are arranged as follows: A tergal row and a spiracular or lateral row the full length of the body; in addition there is a ventral or sternal row in the thoracic region. There is a band of spicules around each segment, but they are not present in any of the succeeding instars. Four pairs of spiracles open upon the surface; there seems to be a fifth pair on the third thoracic segment, although it was impossible to be sure that there was an external opening.

Second Instar

The larva (fig 5, *i*) now has a very different appearance. It resembles neither the first instar nor the full-grown larva. The head

capsule is not so highly chitinized nor are the sensory hairs so prominent as in the first instar; besides, the band of spicules about each segment is missing in this instar as in all succeeding instars.
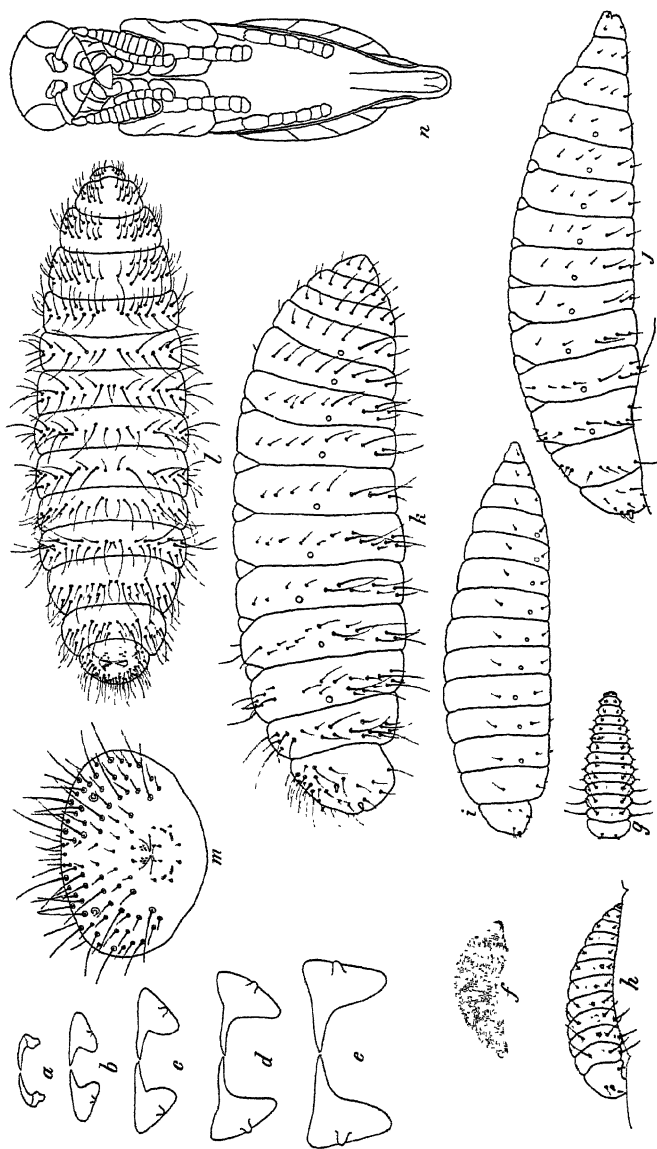
Fig. 5.—*Eurytomocrus iossonatis*: a, Mandibles of first-instar larva; b, same of second-instar larva; c, same of third-instar larva; d, same of fourth-instar larva e, same of full-grown larva; f, egg; g, newly hatched larva (dorsal view); h, larva just before first molt (lateral view); i, second-instar larva (lateral view); j, third-instar larva (lateral view); k, fourth-instar larva (lateral view); l, full-grown larva (ventral view); m, head of full-grown larva showing position of antennae, mouth parts, sensoria, and sensory hairs; n, pupa of female. All greatly enlarged

The head is somewhat intermediate in shape between those of the first instar and the full-grown larva. The head capsule is very feebly chitinized; the head and body are translucent-whitish. The head bears twelve sensory hairs located as follows: A pair just above the

mandibles, a pair between and slightly above the antennae, three on each lateral face, and one just posterior to each mandible. The hairs on one side of the head only are shown in the illustration. The antennae are about twice as long as broad; the mandibles feebly chitinized and triangular in outline. Each of the thoracic segments bears three sensory hairs on each side, and each abdominal segment two hairs on each side, with the exception of the last (tenth) segment, which apparently has only one. The sensory hairs are arranged as follows: A tergal and a lateral row the full length of the body and a sternal row in the thoracic region. There are nine pairs of open spiracles.

The average length of six larvae was 0.88 mm. and the average greatest width 0.233 mm.

THIRD INSTAR

Like the second instar, the third does not resemble the full-grown larva; it (fig. 5, *j*) is slenderer, much less hairy, and the hairs are not so long but are more prominent than in the second instar.

The head and body are colored as in the previous instar. The head bears 16 sensory hairs, arranged as shown in Figure 5, *j*. The hairs on one side of the head only are shown in this sketch. The antennae are about twice as long as wide and proportionately more prominent than in the second instar; the mandibles (fig. 5, *c*) are triangular in outline and feebly chitinized. There are nine pairs of open spiracles. The thoracic segments bear a row of sensory hairs around the entire segment; the first, third, and sixth abdominal segments bear on each side 6 hairs each; the second, fourth, and fifth bear 5 hairs each; the seventh, ninth, and tenth bear 3 hairs and the eighth 4 hairs.

The average length of six larvae was 1.216 mm., and the average greatest width 0.38 mm.

FOURTH INSTAR

At this stage the larva (fig. 5, *k*) is more nearly like the full-grown larva than at any other time. The sensory hairs are about as prominent as in the full-grown larva, but they are not nearly so numerous. The larva is not so slender and pointed as in previous instars, but subcylindrical, tapering only very slightly toward each extremity. There are 13 body segments in addition to the head; the head is somewhat cone-shaped in lateral profile with mouth parts as the point of the cone; the larva is translucent whitish, but brownish in the vicinity of the mandibles; the antennae are about twice as long as broad; the sensory hairs of the head are numerous and quite prominent; the mandibles are triangular and rather strongly chitinized. The body segments are about equal in length with the exception of the last three or four segments of the body; each body segment has a row of hairs around it; those in the thoracic region especially have the appearance of irregular double rows. There are nine pairs of open spiracles.

The average length of eight larvae was 1.63 mm., and the average greatest width 0.50 mm.

FULL-GROWN LARVA

The full-grown larva (fig. 5, *l*) is cylindrical, gradually tapering toward each extremity; it is dirty whitish, often with a dark central area caused by the presence of food in the digestive tract; it is brownish in the mandibular region; the head is almost hemispherical; the mandibles are on the ventral side and point downwards; the antennae are distinctly longer than wide and cone-shaped. The head (fig. 5, *m*) is rather densely covered with coarse sensory hairs of varying length. The labrum is a triangular piece and has a pair of very small sensory hairs near the base, and apparently there are 5 sensory papillae on each side near the angle of the mouth. The ventral mouth parts together form a fused region, which anteriorly is trilobed, corresponding to the component median labial and the two lateral maxillary regions. This fused region (fig. 5, *m*) bears 20 sensory hairs and organs, arranged as follows: 2 sensory hairs or papillae and 2 groups with 3 ring-shaped organs on each lateral or maxillary region and 4 sensory hairs on the median or labial region. This whole fused region is convex. The mandibles (fig. 5, *e*) are triangular in outline, rather heavily chitinized, and dark brown. Each body segment bears one or more irregular rows of long, stout hairs around it, the hairs being most numerous on the anterior and posterior segments. There are nine open spiracles.

The average length of five full-grown larvae was 2.37 mm. and the average greatest width 0.75 mm.

The full-grown larva of this species resembles very closely that of *Ditropinotus aureoviridis* Craw. The distinguishing characteristic of the larva of this species is the dense covering of prominent setae on the front of the head. This is in contrast to the larva of *D. aureoviridis* the head of which is marked with a rather distinct, narrow, centrally located line down the front, which is without setae.

The young larva begins to feed as soon as it pushes its head out of the eggshell and usually continues to feed in this manner for several hours before it completely extricates itself. Apparently it slightly punctures the body wall of the host with its mandibles and then begins to suck up the body fluids. The larvae feed almost continuously, if undisturbed, until nothing remains of the host larva except the empty, dry skin. On the other hand, an individual host larva seems to furnish just enough food for a single parasite. In the field as many as five eggs have been found upon one host. The young larvae, however, are cannibalistic upon occasion and all observations indicate that only one individual ever matures in a single Harmolita cell.

The manner of locomotion is similar to that of other Harmolita parasites. The duration of instars varied from less than a day to 2 or 3 days, varying directly with the temperature of the air and the condition of the host. Thirty-four larvae became full grown in from 4 to 8 days, and the average was 5.58 days during July and August of 1920 and 1921. Two mature larvae remained quiescent 12 and 14 days, respectively, before pupating during the same season. Many larvae of both the first and second generations became full grown in cell slides but died before any of them pupated. Some of these individuals remained in apparently good condition for 17 months and died 4 or 5 months later.

PUPA

Pupation takes place very rapidly. The small number of individuals reared to adults in glass cell slides did not afford an opportunity to observe the process of pupation. No excrement is voided until the larva contracts preparatory to entering the prepupal stage. When entering upon the prepupal stage the first four segments of the abdomen contract equally until each is somewhat less than half its usual length in the full-grown larva. At first the pupa (fig. 5, *n*) is a very delicate brown in color, gradually changing and becoming very dark, approximating the color of the adult insect.

The pupal period of two individuals observed in glass cell slides was 9 and 11 days, respectively. Two female pupae averaged 3.04 mm. in length and 0.78 mm. in greatest width. One male pupa measured 2 mm. in length and 0.58 mm. in greatest width.

ADULT

The adults of this species, both males and females, resemble *Ditropinotus aureoviridis* more closely than any of the other parasites of Harmolita. The female can readily be distinguished from that of *D. aureoviridis* by its dark abdomen and the male by its dark-colored femora and tibiae.

Two generations of this parasite occur normally in Virginia. This seems to hold for Indiana also and is probably true throughout its range, although this can not be stated definitely. The adults of the first generation emerge in June and in Virginia those of the second generation emerge the last week in July. Males normally occur, but the females seem to greatly outnumber them. Females of this species are deuterotokous. The winter is passed as full-grown larvae in the cells of Harmolita. Oviposition is similar to that described for *D. aureoviridis*.[18] The egg, after oviposition, has the same shape as before. Upon dissection, the abdomens of female adults have been found to contain as many as eight well-developed eggs which were apparently ready for deposition.

One individual has been found to deposit as many as 6 eggs in one day. A total of 12 eggs was secured from one individual which had a preoviposition period of 11 days and lived 30 days under as favorable cage conditions as could be provided. In other instances the preoviposition period was 4 days.

The average time required for development from egg to adult during the same season for two individuals was 32 days.

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[18] PHILLIPS, W J , and POOS, F W   LIFE-HISTORY STUDIES OF THREE JOINTWORM PARASITES. Jour. Agr. Research 21 · 405–426, illus. 1921.

A CANKER OF APPLE AND PEAR TREES CAUSED BY GLUTINIUM MACROSPORUM, N. SP. [1]

By S. M. ZELLER

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INTRODUCTION

Fries first erected the genus Glutinium to contain one species, *G. exasperans*, which was considered to belong in the Stilbaceae because of its cylindrical synemaform fruiting bodies which were later found to be pycnidia. In 1894 Starbäck [2] emended the genus and placed it in its rightful position in the Sphaeropsidales. He transferred to the genus the two Fresian species *G. laevatum* (Fr.) Starb. [*Sphaeria laevata* Fr.] and *G. palinum* (Fr.) Starb. [*Phoma palina* (Fr.) Sacc.], *G. exasperans* Fr. being reduced to a synonym of the former. Originally, *G. palinum* was reported on Salix in France and Sweden and *G. laevatum* on *Prunus padus* in Sweden. As far as the present writer can find, no other species has been added to this interesting genus, although McAlpine [3] found one on gumming twigs of peach in 1900. This he referred to *G. laevatum*, although the spores measured only $3.5 \times 1 \mu$.

DESCRIPTION OF FUNGUS ON HOST

In the summer of 1920 the writer found a Glutinium on apple bark in the vicinity of Corvallis, Oreg. This was cultured on potato-glucose agar and sweet clover stems, upon which pycnidia were formed. The material collected was so meager, however, that it was laid aside until more could be had. Late in March, 1924, pear twigs were received from Hood River, Oreg. The tissues around the buds of these were affected by blistery cankers which also extended into the internodes. Cultures from these cankers yielded the same Glutinium observed from apple in 1920.

It was at this time that the writer looked up earlier accessions in the department of botany and plant pathology of the Oregon Agricultural College. He found that in 1912 H. L. Rees, then research assistant of the Oregon Agricultural Experiment Station, had observed the fungus on branches of pear trees in the college orchard and at Salem, Oreg. His complete notes on cultures, inoculation, and the morphology of the fungus have greatly aided in its identification and have made portions of this paper possible. On September 27, 1926, F. D. Bailey discovered this fungus on an apple tree in Marion County, Oreg. This collection is typical of the fungus and is made the basis of the following description.

The cankers with which this organism has been found associated are similar in appearance to winter-injury cankers which have been

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 5
Mar. 1, 1927
Key No. Oreg.-15

42980—27——7   (489)

subsequently invaded by the black-rot organism, *Physalospora malorum* (Peck) Shear, the pycnidia, however, being extremely different in appearance. Rees reports a canker on pear about 5 feet long, extending along the upper side of a scaffold branch (fig. 1), while cankers on apple from 4 to 8 inches long have been observed. The edges of the cankers are usually regular. The dead areas of bark inhabited by the fungus are dry and extend to the discolored wood beneath. The fruiting bodies appear in any region of these dead areas, many times in cracks, or breaking and pushing through the epidermis. When the unbroken epidermis is pulled away the cylindrical pycnidia underneath appear in some instances as vertical columns supporting the raised overlying epidermis. In moist weather the pycnidia under such conditions are somewhat villous and olive green.

FIG. 1.—Side branch of pear tree showing long, narrow Glutinium canker extending along the upper surface. Photograph by H. L Rees

INOCULATIONS

On May 28, 1912, Rees made nine inoculations and as many checks in pear trees. His notes are as follows:

The bark was washed clean with a 1–1,000 solution of mercuric chloride and dried by wiping with a rag with which the solution had been applied. With a sterile scalpel incisions were made and in one a bit of the fungus from a pure culture was inserted. Both of the wounds were then covered with grafting wax by applying with a hot scalpel, the grafting wax having previously been made sterile by boiling. On June 13, 1912, five more inoculations were made in the same manner. In a little more than a month death of the tissue around the point of inoculation was apparent. The epidermis blistered and the affected area became dark in color. By the first of September these areas had become definitely outlined by a crack around the edge of the dead area, which had become sunken. At this time it was seen that all of the 14 inoculations had taken and all checks had remained unaffected and healed over. (Fig. 2.) Four of the inoculated areas were beginning to fruit and by the first of December all showed the fruiting bodies pushing up through the epidermis. They appeared first as black conical bodies and gradually pushed up until about 1 millimeter in height. By the first of January it was seen that spores were escaping from the pycnidia and that the fungus was fruiting in all the inoculated areas. The outlines of the dead areas were slightly irregular, and the dead areas were dry and hard, and extended to the xylem, discoloring it for a depth of about 2 or 3 millimeters. The size of the areas affected from inoculation varied from 2.5 to 3.5 × 3 to 12.5 centimeters. The areas varied from elliptical to circular in shape. Two sets of isolations were made from these cankers, and in every (14) case the same fungus was recovered.

These inoculations show that this Glutinium is at least a wound parasite on pear, capable of considerable growth in apparently healthy bark during the dormant period of the host.   Cankers on apple, which have been seen more recently, show the organism capable of considerable growth in healthy bark.

MORPHOLOGY OF THE FUNGUS

The pycnidia are about 1 to 2 millimeters in height, almost cylindrical, tapering upward, so that the diameters are about 0.6 to 0.8 millimeter at the base and 0.24 to 0.4 millimeter at the ostiole. Seldom are they so short as to appear cone-shaped. (Fig. 3.)   In cross section the pycnidia are circular to slightly elliptical.   In dry weather the pycnidia are covered with a white, flaky exudate giving the black cylinders a whitish sheen. (Figs. 4 and 5.)   The hyaline spores exude at the apex in a flinty, translucent globule. When moist the pycnidia are olive green to black and the globule of spores shows white and glistening.   Rees states that "the pycnidia arise in the collenchyma under the epidermis and in growing push up through the epidermis and carry some of the collenchyma cells up

Fig. 2 —A, pear-bark cankers produced by inoculation with *Glutinium macrosporum*. These inoculations, made in May, showed pycnidia in December of the same year. B, uninoculated lesions made at the same time as the inoculations in A. Photographs by H. L. Rees

with them, so that in the mature pycnidium lines of these cells may be seen arched up into the base of the fruiting body." (Figs. 6 and 7.)   When full grown the pycnidia are almost entirely superficial, only the base being embedded in the outer layer of collenchyma cells and the epidermis peeled back or adhering to the sides of the base. (Fig. 5.)   The pycnidia usually appear singly but may come in groups of two or three.

The base of the pycnidium and about the lower third of the side walls are of a hyaline pseudoparenchyma.   Above this the side walls are composed of very compactly interwoven hyphae with a vertical trend.   The exterior is carbonaceous, gradually becoming hyaline and less compact toward the interior (fig. 8) where the interwoven hyaline hyphae of the wall give rise to the conidiophores within.

The conidiophores branch off from the upward-growing wall hyphae . nearly to the ostiole. In the same manner hyphae from the inner wall at the edges of the ostiole grow out, forming a fringe of protruding, ostiolate hairs, which are sparingly branched and septate.

FIG. 3 —Section of the bark showing pycnidia of *Glutinium macrosporum*. ✕ 1.5. Photographed by H L. Rees

In the base of the pycnidium, hyphae from the walls may form more or less prominent fascicles covered with conidiophores. Where these are prominent the vertical sections of the base appear as divided into locules or pockets. With rejuvenated growth, younger pycnidia may occasionally grow up through older ones, producing a double-walled effect, as shown in Figure 7. The outside of the interior wall in such a case differs only from the newer inside wall by the fact that the interwoven hyphae are more compact and are more carbonaceous. Spores are borne only within the inner cavity and not on the outer layer. The broken wall of the older pycnidium may not extend around the whole younger pycnidium but may be sloughed off or may appear on one side only.

FIG 4.—Pycnidia of *Glutinium macrosporum* enlarged to show cylindrical shape and the whitish exudate on the surface ✕ 4

The spores are acrogenous, ellipsoid, one-celled, hyaline, usually one-guttulate, with a slight thickening of the cell wall at the point of attachment. (Fig. 9.) The spores measure 15 to 28 μ ✕ 8 to 10.5 μ. In certain culture media, as mentioned below, microconidia are formed. These are cylindro-ellipsoid, hyaline, 6 to 10 μ ✕ 2 μ.

CULTURAL CHARACTERISTICS

Cultures of this Glutinium were grown on potato-dextrose agar, prune agar, Lima-bean agar, corn meal, sweet-clover stems, pear and apple stems, and such liquids as beef broth and decoctions of beans and prune. Although the organism showed some growth on all of these media
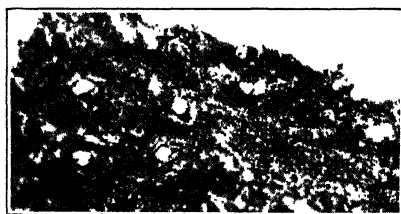
FIG. 5 —Pycnidia breaking through the epidermis of the bark. ✕ 4

the more solid media proved best for luxuriant growth or fruiting. The greatest growth was made on corn meal, Lima-bean agar, and apple, pear, and sweet-clover stems, and on all of these some type of sporulation was produced. Only on sweet-clover stems were typical pycnidia produced in culture. The spores in these, however, were smaller than are found in nature on the host, measuring only 13 to 17 μ ✕ 7 to 8 μ. On the other better media, such as apple

stems, Lima-bean and prune agars, two types of spores were formed. The mycelium appears as a white appressed growth, which takes on a

FIG. 6.—Longitudinal section of a pycnidium of *Glutinium macrosporum.* × 70. Photograph by H.L Rees

FIG. 7.—Longitudinal section of a pycnidium of *Glutinium macrosporum* which has grown up through an older pycnidium, the outer wall of which has been broken away on one side and become more or less incorporated with the wall of the younger pycnidium on the other side. The paraphysate ostiole is prominently shown. × 70. Photograph by H. L. Rees

drab color as minute pustules appear in concentric rings which finally coalesce. Over the surface appear glistening globules composed almost entirely of spores, which are borne on branched conidiophores

on the surface of the mycelial mat. The larger spores are about 18 to 22 $\mu \times 8 \mu$ and the smaller ones 6 to 10 $\mu \times 2 \mu$.

SPORE GERMINATION

Spores from pycnidia on the host readily germinate on a damp microscopic slide in a damp chamber, or in hanging drops of nutrient agar or sterile water.
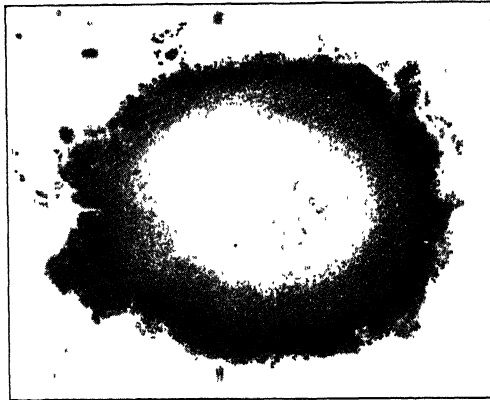
FIG. 8.—Cross section of a pycnidium. × 70 Photograph by H. L. Rees

FIG 9.—Spores of *Glutinium macrosporum* × 500. Photograph by H. L Rees

Germination takes place within 18 hours. One or more germ tubes may develop from a spore. The first indication of germination is the formation of transverse lines across the spore. These are not septa but mark the edges of circular segmentation of the cell wall. These transverse cracks in the cell wall open up, exposing the protoplasmic membrane from which the germ tube or primordial hyphae emerge unobstructed. This is faintly shown in Figure 10. The germ tubes always emerge from these transverse cracks in the cell wall, never from the end of the spore. The germ tubes are

extremely granular and begin septation early. Although repeated attempts to germinate them were made, the microconidia formed in cultures were not seen to do so.

APPLE FRUIT ROT

A fruit rot produced by this organism has not been found in the orchards, but when apples are inoculated a fruit rot is always induced. Ten apples each of three varieties, Newtown Pippin, Jonathan, and Grimes Golden were inoculated, two inoculations and a check being made on each apple. After two weeks small, soft, tan-colored, rotted areas appeared about the inoculations, but the checks remained sound. In six weeks' time the decayed spots had increased to about 4 centimeters in diameter, and had sunken and become darker at the centers, with even definite margins. After nine weeks the apples were well decayed and the organism was reisolated from all the apples. No fruiting of the fungus occurred on the apple fruit. Rees had similar results with five inoculations on two apples. This species of Glutinium therefore is not only a wound parasite, causing a canker of pear and apple, but is capable of causing apple fruit rot.

TAXONOMY AND DESCRIPTION OF FUNGUS

From the study of the morphology of this fungus there is little question that it belongs in the genus *Glutinium* Fr. emend. Starb., and that it is an undescribed species. The only two species previously described have very much smaller pycnidia and spores. Accordingly, the species under consideration will be described as *Glutinium macrosporum*.

Glutinium macrosporum, n. sp.

Pycnidia, olivaceous to black, drying with a white, flaky exudate, cylindrical, often tapering upward, 1 to 2 milli-meters high, 0.6 to 0.8 millimeter diameter below, 0.24 to 0.4 millimeter diameter above, ostiolate; walls of pyc-nidia pseudoparenchymatous below, of compactly interwoven hyphae above, of carbonaceous exterior, and hyaline within; cavity cylindric, lined with simple to slightly branched conidiophores; edges of the ostiole paraphysate; spores ellipsoid, hyaline, one-guttulate, 15 to 28 × 8 to 10.5 μ.

On bark of apple and pear trees in western Oregon. Type collection in Zeller herbarium (No. 6964) and in the herbarium of the department of botany and plant pathology, Oregon Agricul-tural College, Corvallis, Oreg. (No. 4852).

Fig. 10.—Germinating spores of *Glutinium macro-sporum*, showing false septation, which is really a transverse breaking of the wall. ×125. Pho-tograph by H. L. Rees

Because of the size of the spores and the type of pycnidium, this species is closely allied to some species of Macrophoma, but no species in that genus has characters like *Glutinium macrosporum*. The slight branching of the conidiophores suggests Dendrophoma also, but from the other characters it was thought best to refer it to Glutinium.

SUMMARY

A new species of Glutinium has been found occurring on the bark of apple and pear trees in western Oregon. When inoculated into the bark of pear, the fungus proved to be a wound parasite developing a bark canker which extended into the wood beneath. The morphology of the fungus on the host, and cultural and spore germination characteristics on artificial media are described.

A fruit rot of apple is produced by artificial inoculation. The organism is described as *Glutinium macrosporum* Zeller n. sp.

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THE RESULTS OF INOCULATING PINUS STROBUS WITH THE SPORIDIA OF CRONARTIUM RIBICOLA[1]

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INTRODUCTION

During the rather extensive field investigations of white pine blister rust which the Office of Forest Pathology conducted at North Conway, N. H., from 1918 to 1922, large numbers of *Pinus strobus* L. were inoculated with sporidia of *Cronartium ribicola* Fischer. Very little infection resulted from the early inoculations, but a great deal was noted after certain changes in inoculation technique in 1921 and 1922. Preliminary reports on the 1921 inoculations of potted pines (*17*) and on the 1922 inoculations of pines in situ (*7*) have been published. In this latter article various attempts of other investigators (*1, 2, 6, 8, 16, 17*) to infect pines with sporidia artificially are discussed. In the present paper the results of the 1921 and 1922 inoculations of potted pines and the 1925 observations on the 1922 inoculations of pines in situ are reported.

METHODS AND CONDITIONS OF INOCULATION

The methods of inoculation used during the summers of 1921 and 1922 and the conditions under which the inoculations were made, simulated various natural conditions as closely as possible. In both years potted seedlings of *Pinus strobus* ranging in age from 1 to 10 years (most of them under 5 years) and in 1922 pines in situ ranging in age from 5 to 30 years, all of which were growing under natural conditions in the woods, were inoculated with sporidia of *Cronartium ribicola*. All inoculations were made in areas from which the Ribes had been eradicated in 1916. The trees and seedlings inoculated had grown in these areas. This reduced to a minimum the danger of natural infection of the inoculated pines as well as the danger of disseminating the disease from them to near-by Ribes and thence to uninoculated pines. The potted seedling pines had been carefully dug up and transplanted in paraffin paper cups some time before inoculation, so that they had become well established in the pots and had reached a state of physiological equilibrium. The pots had been kept from the time of transplanting to the time of inoculation near a brook in a shady swamp, except in the indicated experiments.

Inoculating material was obtained in 1921 from *Ribes nigrum* L., which was growing in two different localities, and in 1922 from *R. nigrum*, in the same localities, and from *R. odoratum* Wendl. and *R.*

cynosbati (L.) Mill. The inoculum was selected with great care, new generations of telial columns being collected whenever possible.

In both 1921 and 1922 all potted seedlings were inoculated in ice-less refrigerators similar to that described by Hunt (*5*). Some of the pines in situ were inoculated in iceless refrigerators and some on rainy days without them. The temperatures and relative humidities at which the experiments were conducted were recorded by means of frequently checked Fries hygrothermographs.

In 1921 four methods of applying the inoculum to the pine needles were used: (1) An iceless refrigerator was set over a *Ribes nigrum* bush which was producing telia in great abundance. The bush was sprinkled, and potted pine seedlings which had previously been sprayed were placed under it. Throughout the inoculation period droplets of water were present on both the Ribes bush and on the seedling pines. With the exception of this period, the pines were kept more than 900 feet from any Ribes. (2) Telium-bearing Ribes leaves were cut into pieces (Table 1) which were supported by small sticks above the needles of the pines before the teliospores had begun to germinate. (3) Telium-bearing Ribes leaves were cut into pieces (Table 1) and the teliospores were allowed to germinate in Petri-dish moist chambers which were kept on the ground in a cool, moist, shaded spot in the woods for six hours. At the end of that period the pieces of Ribes leaves were supported above the pine needles as in method 2. (4) Telial columns were floated on the surface of water in small glasses which were covered with half Petri dishes and kept under conditions similar to the Petri-dish moist chambers of method 3. At the end of six hours small quantities of germinated telial columns together with the sporidia were removed from the glasses and placed in needle fasicles but not smeared around on the needles. The potted pines inoculated by methods 2, 3, and 4 were sprayed just before and just after the inoculum was applied. They were placed immediately in an iceless refrigerator. For all experiments freshly collected Ribes leaves were used. The inoculum remained on the seedlings for the periods given in Table 1. In some experiments immediately after the removal of the inoculum the seedlings were placed outside in the swamp and in others they were covered with paraffin paper cups to prevent infection from inoculum on other seedlings and were left in the iceless refrigerator in order to continue conditions favorable for infection.

In two experiments the effect of drought and of high humidity upon susceptibility to infection were compared. In these experiments part of the seedlings were exposed to conditions of extreme drought and the others to very high humidity for about a week before inoculation. The seedlings used in the first experiment were placed in half shade and were allowed to wilt frequently, being watered only enough to prevent them from dying. They had almost reached the wilting point when inoculated. The seedlings in the second experiment were set in the shade and the soil in which they were growing was kept almost saturated. They were placed in the iceless refrigerator for several hours before inoculation. After inoculation the seedlings which had received both treatments were placed in the iceless refrigerator. Details of the 1921 experiments are given in Table 1.

TABLE 1.—*Inoculations of potted pines made in 1921 (results to October, 1925)*

| | Conditions of inoculation | | | | First-year seedlings | | Older seedlings | | All seedlings | | |
Date of inoculation (1921)	Pregermination of teliospores [a]	Amount of inoculum	Number of hours inoculum remained on seedlings [b]	Number of hours remained in iceless refrigerator [c]	Number inoculated	Per cent infected	Number inoculated	Per cent infected	Number inoculated	Per cent infected [d]	Per cent living and uninfected [d]
Aug. 12	None	Ribes bush	9¼	9¼	3	0	2	0	5	0	60
Do	do	do	9½	21	0	------	5	0	5	0	60
Do	do	do	14½	21	0	------	6	17	6	17	50
Do	do	do	36	36	8	63	14	14	22	32	59
Aug. 16	do	Leaf fragments 3 to 10 cm. square.	6	42	0	------	4	25	4	25	25
Do	do	do	17	41	3	100	3	33	6	67	33
Do	do	do	24	24	3	67	4	100	7	86	14
Do	do	do	36	36	12	100	26	96	38	97	3
Aug. 25	By floating	Small quantity of telia and sporidia.	24	24	2	50	12	25	14	29	50
Do	None	Leaf fragments ½ to 1 inch square.	21	21	1	100	18	44	19	47	37
Aug. 26	do	Leaf fragments 1 inch square.	6	6	0	------	5	40	5	40	60
Do	do	do	9	9	0	------	4	25	4	25	75
Do	do	do	9	51	1	100	4	50	5	60	40
Do	do	do	42	42	18	78	11	82	•29	79	10
Aug. 24	In Petri dish	do	23½	23½	3	67	9	78	12	75	8
Aug. 29[f]	do	do	24	24	4	50	7	57	11	55	9
Do[g]	do	do	24	24	2	100	8	25	10	40	40
Do[f]	By floating	Small quantity telia and sporidia.	24	24	0	------	6	67	6	67	17
Do[g]	do	do	24	24	0	------	9	67	9	67	33

[a] In all experiments in which teliospores were pregerminated (germinated before being placed in iceless refrigerator) the germination period was six hours.
[b] Some seedlings were placed outside immediately after the removal of the inoculum while others were covered with paraffin paper cups and kept in the iceless refrigerator.
[c] When the seedlings were removed from the iceless refrigerator they were placed outside in the swamp.
[d] It will be noted that in some cases the percentages in the last two columns do not total 100. This is because some of the seedlings died before it was possible to determine either the presence or the absence of infection.
[e] Includes five plants on which inoculum remained only six hours but which were accidently mixed with others.
[f] Exposed to extreme drought for about one week before inoculation.
[g] Exposed to high humidity for about one week before inoculation.

In 1922 the methods of inoculation were very similar to Nos. 2 and 3 which were used in 1921. After collection, the telium-bearing Ribes leaves were placed in a refrigerator for at least 12 hours, and the leaves were then cut into quarters or halves according to size. In part of the experiments the telial columns were ungerminated and in the others they had been allowed to germinate in Petri-dish moist chambers which were kept in the laboratory for six hours before being carried to the above-mentioned swamp. The pieces of telium-bearing Ribes leaves were lodged in the axils of the pine needles where they were held loosely in place by means of hairpins made of fine wire (*7, fig. 2*) instead of by means of small sticks as in 1921. The seedlings were sprayed before and after inoculation and were placed immediately in iceless refrigerators, as in 1921. The inoculum was left in place for varying periods. (Table 3.) After the removal of the inoculum some seedlings were immediately placed outside in the swamp, while others were placed for varying periods in a second iceless refrigerator instead of being covered by paraffin paper cups in the same iceless refrigerator as in 1921. In both 1921 and 1922 all

inoculating materials were buried just as soon as they were removed from the seedlings.

In both years after the inoculated seedlings were removed from the iceless refrigerators they were left in the swamp for a few days where they were shaded all day. They were then placed for a while in a place where they received half shade. Finally they were planted in a garden where the pots were buried in the soil. The bottoms of the pots were perforated or removed before planting. The seedlings were watered frequently until they became established. Uninoculated seedlings were planted in the garden beside the inoculated ones for the purpose of securing additional evidence that the infections on the inoculated seedlings had resulted from artificial rather than natural inoculations.

RESULTS OF THE 1921 INOCULATIONS OF POTTED PINES

The results of the 1921 inoculations are reported in one section and those of the 1922 inoculations in the section immediately following, because the 1922 results did not confirm those for 1921 in certain details.

Data were taken on the 1921 inoculations in November, 1921 (*17*), in July, 1922, in June, 1923, and in June and October, 1925. The unavoidably long intervals between observations make it probable that some infected seedlings died without recognition of the infection. No seedling was classified as infected unless definite evidence of infection was obtained. The presence of infection was determined by the external symptoms and in certain doubtful cases by sectioning and staining by the method described by Colley (*3*).

The first external symptoms of white pine blister rust infection are the characteristic yellow spots in the needles described by Clinton (*2*) and illustrated for the first time in color in Plate 1 of this article. These had appeared in the needles of some of the seedlings three months (*17*) after inoculation. Subsequent symptoms of infection were the stunting of growth, the swelling of the stem, the discoloration of the stem, the curving of the stem, abnormally bushy growth, and the production of adventitious buds. Usually a seedling showed more than one of these symptoms.

For each experiment performed in 1921 the percentage of inoculated seedlings which showed evidence of infection between the time of inoculation and October, 1925, is given in Table 1. No infection was noted on any of the uninoculated seedlings, although it was present in every series of inoculated seedlings with the exception of two. It occurred even when the inoculum was removed and the seedlings were set out of doors in the swamp six hours after ungerminated telial columns were suspended over them in the iceless refrigerator. The purpose of this experiment was to ascertain whether infection may occur in nature at the time of heavy dews. The results seem to indicate that such infections would be possible. The minimum time needed for white pine blister rust infection is made by this result to appear materially shorter than the 17½ hours previously suggested (*12*).

EXPLANATORY LEGEND FOR PLATE 1

Seedling of *Pinus strobus*, showing the yellow needle spots characteristic of white pine blister rust infection
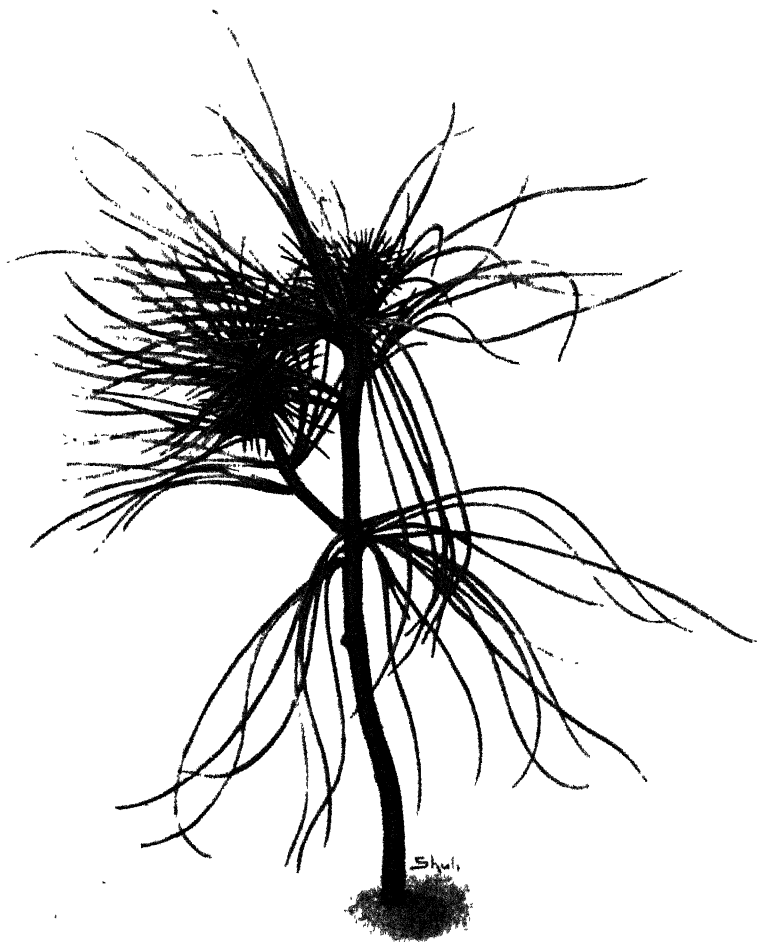
It is well known that during some years blister rust infections on pines are abundant, while during others in which Ribes infections are seemingly just as abundant there is little or no infection of pines. The fact that in one experiment sporidia were observed three hours after teliospores were placed in conditions suitable for germination (*14*), that sporidia began to germinate one hour after being placed under suitable conditions (*17*), that the infection period is sometimes as short as recorded in Table 1, and that dried sporidia may sometimes remain viable for 26 hours (*14*), indicate that the physical factor requirements of the fungus are less exacting than has been supposed. It is true that no tests have been made to determine whether the sporidia which were observed after three hours were mature enough to germinate, and none have been made to determine whether the sporidia found viable after long periods of drying are still capable of infecting pines. The information available
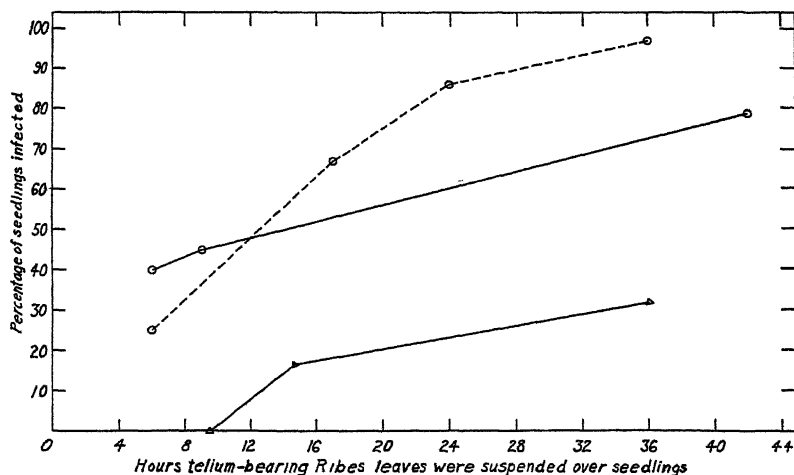
FIG. 1.—Relationship in the 1921 experiments between the amount of infection and the length of time the inoculum remained on the seedlings. Each curve represents a different experiment. The teliospores were ungerminated when suspended over the seedlings

seems, nevertheless, to warrant the suggestion that the internal condition of the pines may be quite as important as external physical factors in explaining the infrequence of pine infections.

In the 1921 experiments the amount of infection tended to increase directly with the length of time the inoculum remained on the seedlings. (Fig. 1 and Table 1.) This relationship was less evident for the 1922 experiments than for those of 1921; and in the 1921 experiments less for first-year seedlings than for older ones. It is probable that the longer the teliospores were suspended over the seedlings the more sporidia were produced and hence the greater the chances of infection. Of course it is possible that the observed relationship was purely accidental and resulted from the difference in susceptibility of individual trees or individual needles. The fact that the relationship for the first-year seedlings was less evident than for the older ones may possibly be explained on the ground of the greater susceptibility of the first-year seedlings, that is, the first-year seedlings were so susceptible that they became infected regardless of the

length of time that the inoculum remained upon them. At any rate in comparable 1921 experiments 77 per cent of the first-year seedlings became infected, whereas only 58 per cent of the older ones (most of which were over 3 years old) became infected. Clinton (*2*) found that a larger percentage of his 1-year-old than of his older seedlings became infected.

The two experiments conducted for the purpose of determining the comparative effect of exposure of seedlings of *Pinus strobus* to drought and to high humidity for some time before inoculation upon their subsequent susceptibility to infection with sporidia of *Cronartium ribicola* were inconclusive. Seedlings exposed to drought as well as those which had been kept at a high relative humidity became
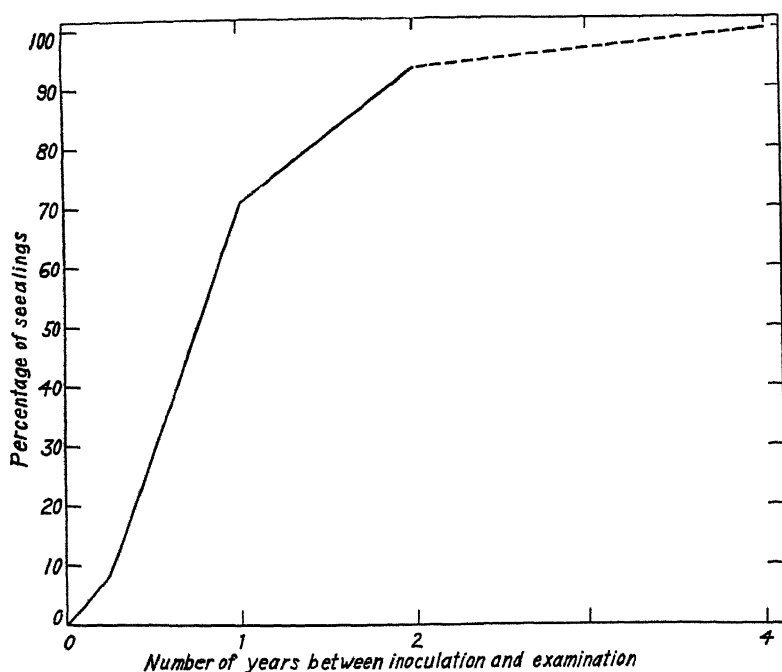
Fig. 2.—Rate at which external symptoms of infection appeared on the infected seedlings in the 1921 inoculations. No data were taken three years after inoculation

infected, and there was not enough difference between the amounts of infection to show which condition made the seedlings more susceptible. (Table 1.) However, in October, 1925, it was found that in both experiments fewer seedlings were living and uninfected among those which had been exposed to drought just before inoculation than among those kept at high relative humidity. Of course it is possible that the exposure to drought and not rust infection may have been responsible for some of these deaths.

Some infections resulted from all four methods of inoculation. (Table 1.) More infections were obtained with sporidia produced by teliospores which had been germinated by "floating" upon the surface of water than in previous attempts by this method, and

more than by inoculation at the same time and under the same conditions with sporidia produced in moist chambers. It is likely that the reason for the greater number of infections is that more sporidia are produced by "floating" than by germination in moist chambers (*13*). In earlier tests without iceless refrigerators few infections resulted from inoculations with sporidia produced by "floating."

The rate at which external evidences of infection from the 1921 inoculations became visible in the infected seedlings is shown in Figure 2. Most of the infections which had occurred had become visible within two years after inoculation. One year after inoculation about 14 per cent of the infected seedlings (fig. 3) had died and
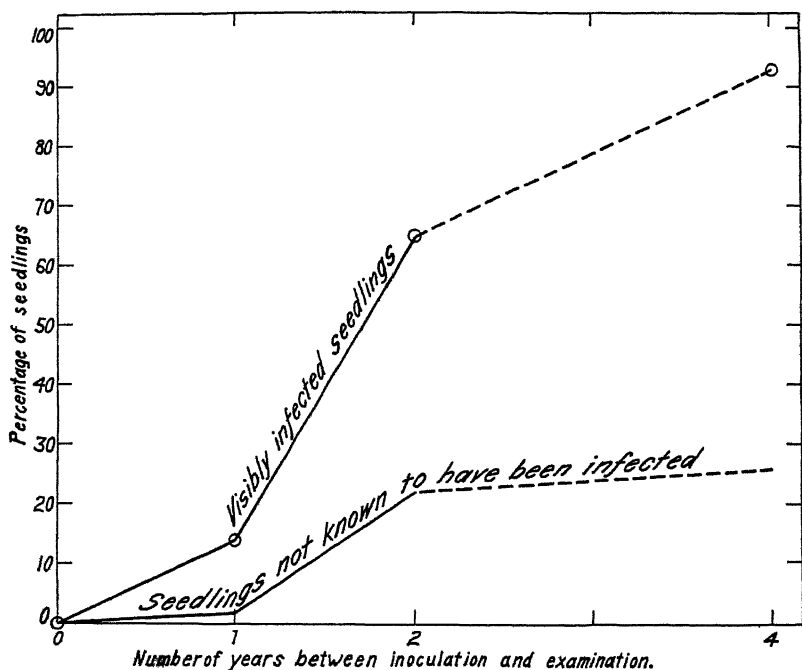
Fig. 3.—Death rate of the visibly infected seedlings inoculated in 1921 and of those not known to have been infected

about one-third of them showed external symptoms of blister rust in their woody parts.

Figure 3 gives a comparison between the rate of death of the seedlings known to have been infected and of those not known to have been infected. Without doubt some of the latter were really infected, since some of them had died and disappeared before they were examined for evidences of infection. If all infections had been detected before death the differences between the two lines in Figure 3 would probably have been still greater. No leaf spots were detected on some infected seedlings while leaf spots, but no further evidence of infection were found on other seedlings. These latter were not classified as infected.

RESULTS OF THE 1922 INOCULATIONS OF POTTED PINES

Data were taken on the 1922 inoculations of potted pines in June, 1923, and in June and October, 1925. The results are given in Tables 2 and 3. The unavoidably long intervals between observations made it inevitable that a great many seedlings should die without either positive evidence of infection or of lack of it having been obtained. There was no consistent relationship between these deaths and the length of time the inoculum remained on the seedlings or between the deaths and the age of the seedlings. There was, however, a tendency for larger percentages of the younger than of the older seedlings to die.

TABLE 2.—*Amount of infection on potted seedlings of different ages (1922 inoculations)*

TELIOSPORES UNGERMINATED WHEN SUSPENDED OVER SEEDLINGS

	Age of seedlings									
	11th year	8th year	7th year	6th year	5th year	4th year	3d year	2d year	1st year	Total
Number of seedlings inoculated		1	1	8	10	13	10	15	3	61
Percentage of seedlings dead, 1923		100	0	13	20	15	30	40	33	26
Percentage of remainder with doubtful results [a]			0	14	25	45	57	55	50	40
Percentage of remainder infected			0	43	50	45	14	11	0	31

TELIOSPORES GERMINATED WHEN SUSPENDED OVER SEEDLINGS

Number of seedlings inoculated	2	3	6	19	38	42	28	82	9	229
Percentage of seedlings dead, 1923	0	0	0	0	5	5	29	34	43	24
Percentage of remainder with doubtful results [a]	0	0	50	21	22	40	30	52	1C0	38
Percentage of remainder infected	100	67	16	32	42	38	20	24	0	31

[a] Most of these seedlings died between June, 1923, and June, 1925.

TABLE 3.—*Amount of infection on potted seedlings on which inoculum had been suspended for different periods (1922 inoculations)*

TELIOSPORES UNGERMINATED WHEN SUSPENDED OVER SEEDLINGS

	Inoculum in place								
	5 to 6 hours	9 to 10 hours	11 to 12 hours	18 to 19 hours	24 hours	27 hours	30 hours	48 hours	68 hours	Total
Number of seedlings inoculated			18	14	14		7	8		61
Percentage of seedlings dead, 1923			22	21	43		43	0		26
Percentage of remainder with results doubtful [a]			50	54	13		50	24		40
Percentage of remainder infected			21	27	50		25	38		31

TELIOSPORES GERMINATED WHEN SUSPENDED OVER SEEDLINGS

Number of seedlings inoculated	54	24	42	35	44	10		11	9	229
Percentage of seedlings dead, 1923	17	13	26	20	19	20		9	22	24
Percentage of remainder with results doubtful [a]	47	47	29	43	17	37		60	43	38
Percentage of remainder infected	29	29	32	25	49	13		20	29	31

[a] Most of these seedlings died between June, 1923, and June, 1925.

In 1922 some infection occurred in every experiment. Contrary to the 1921 results, the amount of infection seemed to bear no consistent relationship to the length of time in which the inoculum remained in place. Sporidia from teliospores which were ungerminated when suspended over the seedlings produced on the whole as much infection as those from teliospores which were pregerminated. The infection percentages of seedlings which were placed out of doors after the removal of the inoculum were approximately the same as those of seedlings upon which the inoculum had remained for an equal length of time but which were placed in a second iceless refrigerator after its removal. However, the one 1921 experiment in which like comparisons were possible indicated a very decided advantage for continuation in the iceless refrigerator after the removal of the inoculum.

Seedlings of all years except first-year ones became definitely infected, contradicting the 1921 results that seedlings of the current year were more readily infected than older ones. This fact, together with the fact that two-thirds of the seedlings infected in 1921 had died within two years of infection and 90 per cent within four years, helps to account for the absence of 1 to 15 year old seedlings (*4*) in stands infected with blister rust at Waterford, Vt., and at other places.

SUPPLEMENTARY OBSERVATIONS ON THE 1922 INOCULATIONS OF TREES IN SITU

The conditions and methods of inoculating 5 to 30-year old pines in situ in the summer of 1922, and the results of these inoculations which had appeared by September, 1924, have been published (*7*). In 1925 only a portion of the 1922 inoculations could be found because during logging operations some of the inoculated trees had been broken down and destroyed and labels had been removed from others. For this reason the results given below are less representative than they otherwise would have been. From Table 4, it can be seen that there is no real evidence to support the suggestion (*7*) previously made that infection occurred less readily within moist chambers than outside of them on rainy days. For the different experiments the percentage infected ranged from 0 to 90 of the inoculated branches. On these branches the percentage of inoculated twigs infected ranged from 0 to 100. Even on the infected branches only 24 per cent of the twigs were infected.

TABLE 4.—*Results of the 1922 inoculations of 5 to 30 year old pines growing naturally in the woods (observations made in October, 1925)*

Place of inoculation	Number of branches inoculated	Number of branches infected	Per cent of branches infected	Number of twigs inoculated	Number of twigs infected	Per cent of twigs infected
In moist chambers	*a* 126	39	31	823	103	13
Without moist chambers	*a* 40	14	35	374	37	10
Total	*a* 166	53	32	1,197	140	12

a As a rule several twigs were inoculated on each branch. The figures in column 2 differ from those reported for the September, 1924, (*7*) observations because a great many inoculated branches were destroyed during logging operations.

Various symptoms of blister rust were present on the infected twigs in 1925. These consisted of discolorations, swelling, constriction, and deformation (curving) of the twigs, and pycnial and aecial scars. Frequently more than one symptom was present on the same twig. Many of the cankers had girdled the infected twigs and killed the distal parts of them. Many of the killed parts had been invaded by secondary fungi. One small tree about a foot high which was infected in every twig had been killed before June, 1925. In June and September, 1923, practically the only symptoms of blister rust infection observed were the characteristic needle spots (*2*).

No symptoms of infection have been observed on the nine trees of *Pinus resinosa* Ait., which were inoculated with sporidia of *Cronartium ribicola* by the same method as the *P. strobus* trees (*7*) both with and without moist chambers. Clinton (*2*) also got negative results with this species.

RELATION OF TEMPERATURE AND RELATIVE HUMIDITY TO INFECTION OF PINES

So many factors influence the infection of *Pinus strobus* by the sporidia of *Cronartium ribicola* that it is often impossible to determine whether the observed temperatures and relative humidities or some undetected causes are responsible for the failure of inoculations. Temperature and relative humidity influence the production and the germination of the sporidia as well as the actual entrance into the pine needles.

Teliospores will germinate and produce sporidia when floating upon the surface of water and in moist chambers at temperatures of 55° to 80° F. Spaulding (*10*) also found them germinating upon melting snow.

Sporidia germinated in iceless refrigerators where the average relative humidity was 99 per cent and the average temperature 60° F. They also germinated readily on moist slides in a cellar where the temperature ranged from 60° to 70°. Sporidia which had been dried and exposed for 26 hours (*14*) to temperatures of 58° to 62° and 60° to 62° and relative humidities of 80 to 85 per cent and of 74 to 84 per cent, respectively, were able to germinate, but no tests were made to determine whether they could infect pines.

In 1922 pines in situ (*7*) became infected within and without iceless refrigerators at high relative humidities and at rather low temperatures. In 1921 (*17*) potted pines became infected at an average relative humidity of 94 per cent and an average temperature of 65° F. They also became infected when the average relative humidity was 88 per cent. In 1922 some potted pines became infected in every experiment. The relative humidities ranged from 74 to 99 per cent and the temperatures from 51° to 72°. The relative humidity was above 90 per cent most of the time, and it was commonly above 95 per cent. From these results some, but not the extreme, ranges of temperatures and the relative humidities which permit infection can be ascertained. The temperatures and relative humidities which prevent infection are not yet known.

RELATIVE SUSCEPTIBILITY OF 1, 2, AND 3 YEAR OLD NEEDLES OF
PINUS STROBUS

Clinton (*2*) and the writers (*7, 17*) stated that both the first and second year needles of *Pinus strobus* may become infected, but they gave no data on the relative susceptibility of needles of different ages. In an attempt to get some definite information on this point in 1922, 60 inoculations were made on needles of known ages on older trees. Two per cent of these inoculations were made on 1920 needles, 18 per cent on 1921 needles, and 80 per cent on 1922 needles. None of the inoculations on 1920 needles, 18 per cent of those on the 1921 needles, and 19 per cent of those on the 1922 needles developed leaf spots. None of the inoculations on 1920 needles, 9 per cent of those on 1921 needles, and 8 per cent of those on the 1922 needles resulted in twig cankers. These results agree with the statement previously made that 1921 and 1922 needles seemed of approximately equal susceptibility (*7*).

One hundred and twenty-seven other cankers resulting from inoculations made on the pines in situ in 1922, were examined in October, 1925, in order to ascertain the age of wood upon which they occurred. Ninety-five per cent of these cankers were on 1922 wood and 5 per cent on 1921 wood. These results confirm the above statement that both 1 and 2 year old needles are susceptible. They do not, however, give any information on the relative susceptibility of 1921 and 1922 needles, for no record was kept of the relative percentages of the 1921 and 1922 needles originally inoculated. The most that can be said is that no indication was encountered that 2-year-old needles of *Pinus strobus* are more liable to infection than 1-year-old needles.

External symptoms as well as sectioning indicate that the 1920 as well as the 1921 needles of potted seedlings inoculated in 1921 were susceptible to infection. The 1921 and 1922 needles of potted seedlings inoculated in 1922 were also susceptible. However, no 1922 seedling became definitely infected. The fact that both 1-year-old and 2-year-old needles are susceptible increases the chances of infection.

Approximately 40 per cent of the 1922 inoculations of branches of pines in situ which had spotted needles in June, 1923, developed no further symptoms of infection between that date and October, 1925. None of the spotted needles were sectioned because a maximum number of twig infections were desired, and it is entirely possible that some of the needle spots were caused by something besides blister rust infections. Approximately one-tenth of the potted seedlings inoculated in 1921 whose needles became spotted developed no further symptoms of rust infection. Sections made by the method described by Colley (*3*) showed that the needles of one of these seedlings were definitely infected. Also about one-quarter of the potted seedlings inoculated in 1922 which developed leaf spots showed no further evidence of infection. Spaulding (*11*) stated that the relative susceptibility of the different white pines to *Cronartium ribicola* seems to be associated with the length of time the needles persist and that the needles of *Pinus strobus* persist for two to three years. He concluded, "with *P. strobus* there is a chance that the older infected leaves may drop before the fungus reaches the bark of the twig." Spaulding's

theory probably accounts for the presence of rust symptoms in needles and their subsequent absence in twigs in some of the writers' experiments, but as spots resembling those caused by blister rust are sometimes present in needles which are not infected other causes may account for some of them. For this reason it is necessary to section and stain spotted pine needles in order to be sure that the needle spots are white pine blister rust infections.

On the other hand, a large number of infections occurred on the inoculated twigs of the pines in situ without needle spots having been observed on the dates of inspection. About half of the potted seedlings which eventually showed symptoms of blister rust developed no leaf spots which were visible at the times of inspection.

RELATIVE VIRULENCE OF SPORIDIA FROM DIFFERENT RIBES HOSTS

It has been stated that by September, 1924, none of the sporidia from *Ribes cynosbati* had produced external symptoms of infection on the trees in situ inoculated with them in 1922, but that sporidia from *R. odoratum* and those from *R. nigrum* from two localities had caused infections with typical external symptoms (7). In 1925 it was found that sporidia from *R. cynosbati* had also caused some infections on the inoculated trees. Inoculations with sporidia from different hosts were not made simultaneously. For this reason and because so many inoculated twigs and branches were lost in the logging operations mentioned above, it is impossible to compare directly the infection precentages of the sporidia from the different hosts. The indications are, however, that the infection percentages were influenced mainly by factors other than the hosts upon which the sporidia were produced.

Sporidia from *Ribes nigrum* only were used in the 1921 inoculations. Sporidia from *R. cynosbati*, from *R. odoratum*, and from *R. nigrum* collected in two localities all caused some infection on potted pines inoculated in 1922. The inoculations with sporidia from the different Ribes hosts were not made simultaneously, but the results are presented in Table 5 for what they are worth. Comparisons of the infection percentages seem to indicate that sporidia from *R. cynosbati* were less virulent than those from the other two hosts but comparisons of the percentages of healthy seedlings remaining in October, 1925, seem to indicate that sporidia from all three hosts were of approximately equal virulence.

TABLE 5.—*Relative virulence of sporidia of Cronartium ribicola from different species of Ribes*

Source of sporidia	Number of seedlings inoculated	Per cent infected [a]	Per cent healthy [a]
Telia from Ribes cynosbati [b]	27	19	30
Telia from Ribes odoratum [b]	24	33	29
Telia from Ribes nigrum [b] (North Conway, N. H.)	51	39	28
Telia from Ribes nigrum [b] (Twin Mountain, N. H.)	83	30	34
Do. [c]	45	31	29

[a] These percentage figures do not total 100 because a great many of the seedlings died before the presence or absence of infection had been determined.
[b] Teliospores pregerminated when suspended over seedlings and placed in iceless refrigerator.
[c] Telia ungerminated when suspended over seedlings and placed in iceless refrigerator.

This would mean that if *Ribes odoratum* and *R. cynosbati* produced as many sporidia as *R. nigrum* (*15*), bushes of those species would be just as dangerous to near-by pines as those of *R. nigrum*.   General field experience corroborates this suggestion.   There is, however, some indication which needs more definite proof that sporidia from *R. nigrum* are more tolerant of drying than those from other species (*14*).

TIME ELAPSING BETWEEN INOCULATION AND PRODUCTION OF PYCNIA AND AECIA

In October, 1925, 140 cankers on pines in situ inoculated in 1922 were examined for evidences of the production of pycnia and aecia. Only 14, or 10 per cent, of these had produced pycnia and only 4, or 3 per cent, had produced aecia.   This makes the period elapsing between infection and the first production of aecia approximately three years.   The first pycnia were produced at some earlier date. For most of the cankers the period between infection and the production of pycnia and aecia will be more than three years.   Most of the infected potted seedlings died without producing either pycnia or aecia.

Spaulding (*9*) summarized the known data on the incubation period of *Cronartium ribicola* on pines.   Artificially infected seedlings of *Pinus strobus* produced pycnia 5 to 6 months (*2*), 10 months (*6, 16*), and 13 months (*8*) after inoculation.   The incubation periods for blister rust in these seedlings, most of which were inoculated in greenhouses, were naturally shorter than those in the writers' out-of-doors experiments.   Tubeuf (*16*) observed aecia 32 months after inoculation, which is approximately the same length of time as that observed by the writers.

SUMMARY

Sporidia from teliospores which had been allowed to germinate in moist chambers for six hours before being suspended over potted seedlings in an iceless refrigerator caused on the whole neither more nor quicker infection than sporidia from teliospores which were ungerminated when placed in the iceless refrigerator.

Infections occurred at rather high relative humidities and at medium temperatures.   The extreme temperature and relative humidity ranges suitable for infection have not been determined.

Sporidia from *Ribes cynosbati, R. odoratum*, and *R. nigrum* were able to infect both the potted seedlings and the pines in situ.   Sporidia from other Ribes were not tested.

External symptons as well as sectioning showed that both 1 and 2 year old needles of *Pinus strobus* are susceptible to sporidia of *Cronartium ribicola*.   So far as ascertained, the younger and older needles are approximately equally susceptible.   Cankers developed on wood which was formed in the same season in which inoculations were made, as well as on wood which was formed in the preceding season.

External evidences of infection confirmed by sectioning were present in the needles three months after inoculation.

Potted seedlings of the current year in certain experiments, as well as older ones, became infected.

Some of the infected potted seedlings had died one year after inoculation. Mortality was higher in inoculated potted seedlings which were known to have been infected than in inoculated ones on which no infection was visible.

Only a small percentage of the infections had produced either pycnia or aecia three years after inoculation.

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A MORPHOLOGIC AND BIOMETRIC COMPARISON OF CRONARTIUM RIBICOLA AND CRONARTIUM OCCIDENTALE IN THE AECIAL STAGE [1]

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INTRODUCTION

Ever since the piñon blister rust, *Cronartium occidentale* Hedgcock, Bethel, and Hunt (*5*),[3] was discovered to be widespread in certain sections of the Western States, the question has been raised as to whether it was really distinct from the white pine blister rust, *Cronartium ribicola* Fischer. The former is relatively innocuous, attacking—so far as is known—only the nut pines; but the latter is a particularly destructive parasite infecting the valuable 5-needled pines. The uredinial and telial stages of both forms occur on members of the genus Ribes. A recent study (*3*) has shown that the urediniospores of the two species are very close together biometrically but fairly consistently different, and that the size and shape of the spores are reasonably reliable criteria for diagnosis. The purpose of the present paper is to submit the evidence of morphologic and biometric differences in the aecial stages. These differences appear to be both significant and easily recognized.

MATERIAL

The chief source of material was the office collection. In addition, specimens which had been specially preserved in formalin-alcohol (6 c. c. full strength [37 per cent] commercial formaldehyde to 94 c. c. of 70 per cent alcohol) and specimens fresh from the field were used. Slides were made from these preserved and fresh specimens; and slides which had been prepared in previous work on the morphology and cytology of *Cronartium ribicola* (*1*) were carefully restudied.

Herbarium material only was selected for the biometric study of the aeciospores and peridia. For each species apparently representative specimens, with as wide a locality and time of collection range as possible, were chosen. Most of the *Cronartium ribicola* material was on the one host *Pinus strobus* L., although there were three specimens on *P. monticola*, but the *C. occidentale* selections were about equally divided between *P. edulis* Engelm. and *P. monophylla* Torr. and Frem. The collection data are given in full in Table 1.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 6
Mar. 15, 1927
Key No. G-568

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TABLE 1.—*Biometric data on aeciospores of Cronartium ribicola and C. occidentale, based on 100 spores from each specimen*

| Host | Locality | Date collected | Length | | Width | | Mean wall thick-ness | Ratio· Mean length, mean width |
			Mean	Stand-ard devia-tion	Mean	Stand-ard devia-tion		
Cronartium ribi-cola:			*μ*		*μ*		*μ*	
Pinus strobus.	Dunraven, N. Y._____	May 20, 1915	24. 4	2. 3	17. 9	1. 5	3. 36	1. 36
Do_____	East Randolph, Vt_____	May 18, 1916	25. 1	2. 0	18. 1	1. 5	3. 04	1. 38
Do_____	Woodstock, Vt_____	_____do_____	24. 0	2. 0	17. 7	1. 4	2. 98	1. 35
Do_____	Kittery Point, Me_____	May —, 1917	24. 8	2. 0	17. 9	1. 5	2. 82	1. 38
Do_____	_____do_____	1917	25. 2	2. 2	19. 1	1. 4	3. 69	1. 31
Do_____	_____do_____	May 18, 1917	24. 8	2. 9	18. 5	1. 9	_____	1. 34
Do_____	Exeter, N. H._____	May 24, 1917	25. 6	1. 8	19. 7	1. 3	3. 69	1. 29
Do_____	Stratham, N. H._____	May —, 1917	25. 9	1. 9	19. 4	1. 4	3. 34	1. 33
Do_____	Marlow, N. H._____	June 14, 1917	24. 3	2. 9	19. 1	1. 8	4. 18	1. 27
Do_____	Gloversville, N. Y_____	Sept. —, 1917	23. 9	2. 4	14. 9	1. 9	_____	1. 60
Do_____	Kingston, Mass._____	Mar. 22, 1918	23. 3	2. 4	17. 7	1. 5	3. 10	1. 32
Do_____	Brunswick, Me._____	Apr. 30, 1918	24. 0	2. 0	18. 7	1. 4	3. 20	1. 28
Do_____	Halifax, Mass._____	May 13, 1918	22. 6	1. 8	18. 2	1. 4	3. 00	1. 24
Do_____	Maine._____	May —, 1919	24. 1	1. 9	19. 0	1. 5	3. 24	1. 27
Do_____	Block Island, R. I.____	May —, 1920	24. 0	2. 6	17. 7	1. 8	_____	1. 35
Do_____	Portland, Me._____	June —, 1920	24. 0	2. 0	18. 0	2. 9	_____	1. 33
Do_____	North Conway, N. H___	_____do_____	24. 9	4. 1	16. 7	2. 1	_____	1. 49
Do_____	Topsfield, Mass._____	Mar. —, 1921	22. 3	2. 1	18. 1	2. 0	_____	1. 23
Do_____	_____do_____	Mar. 27, 1921	24. 0	2. 2	18. 7	1. 5	3. 17	1. 28
Do_____	Block Island, R. I.____	Apr. 6, 1921	22. 0	1. 9	17. 2	1. 5	2. 81	1. 27
Do_____	South Deerfield, N. H__	May —, 1922	23. 7	2. 2	18. 3	1. 8	3. 23	1. 29
Do_____	Vancouver, B. C._____	Mar. —, 1922	22. 4	2. 8	16. 8	1. 6	2. 97	1. 33
Pinus monti-cola.	North Vancouver, B. C.	June —, 1922	25. 9	2. 2	19. 6	1. 6	4. 29	1. 32
Do_____	_____do_____	June 7, 1922	26. 3	2. 0	20. 6	1. 6	[1] 4. 50	1. 27
Do_____	Vancouver, B. C._____	June 9, 1922	24. 3	2. 0	19. 2	1. 6	[1] 4. 17	1. 26
Constants com-puted from speci-men means.	_____	_____	24. 2	1. 11	18. 3	1. 14	3. 41	[2] 1. 32
Cronartium occi-dentale:								
Pinus edulis___	Bayfield, Colo_____	May 19, 1918	27. 2	2. 6	18. 9	1. 8	4. 01	1. 43
Do_____	Trimble Hot Springs, Colo.	June 23, 1918	25. 7	2. 1	18. 6	2. 2	3. 48	1. 36
Do_____	Mancos, Colo_____	June 28, 1918	26. 5	2. 3	19. 3	2. 0	3. 51	1. 37
Do_____	_____do_____	June 29, 1918	27. 4	3. 0	20. 0	2. 1	3. 71	1. 37
Do_____	Mesa Verde National Park, Colo.	July —, 1918	25. 8	2. 6	19. 0	2. 4	_____	1. 35
Do_____	Glenwood Springs, Colo.	May 28, 1919	25. 7	2. 4	18. 8	1. 4	3. 62	1 36
Do_____	Bayfield, Colo_____	Oct. 15, 1920	28. 8	3. 3	19 4	2. 4	4. 29	1. 48
Do_____	Mancos, Colo_____	Oct. 16, 1920	23. 6	2. 3	18. 5	1. 8	3. 64	1. 27
Pinus mono-phylla.	Minden, Nev_____	May —, 1920	26. 7	2. 7	16. 8	2. 7	_____	1. 59
Do_____	_____do_____	May 20, 1920	28. 1	3. 3	20. 9	2. 4	4. 39	1. 34
Do_____	_____do_____	May 22, 1920	28. 5	2. 7	20. 6	1. 6	4. 75	1. 38
Do_____	_____do_____	May 30, 1920	27. 1	2. 9	18. 9	1. 6	3. 97	1. 43
Do_____	Bridgeport, Calif_____	May 31, 1920	27. 8	3. 0	18. 3	2. 0	3. 31	1. 51
Do_____	Walker Canyon, Calif__	_____do_____	27. 1	2. 5	18. 0	1. 8	3. 78	1. 50
Do_____	Minden, Nev_____	June —, 1920	27. 1	2. 9	18. 2	2. 2	_____	1. 49
Do_____	_____do_____	Aug. —, 1920	26. 3	2. 6	18. 9	2. 6	_____	1. 39
Do_____	Bethel Collection_____	Sept. —, 1920	26. 4	2. 8	19 0	2. 2	_____	1. 39
Do_____	Bridgeport, Calif_____	Sept. 12, 1920	28. 1	2. 5	20. 5	2. 5	4. 03	1. 37
Do_____	Sweetwater, Nev_____	Sept. 14, 1920	27. 0	2. 9	19. 6	2. 2	3. 40	1. 37
Do_____	Carter's Station, Nev___	June —, 1921	25. 3	3. 4	18. 1	2. 3	_____	1. 39
Do_____	Bridgeport, Calif_____	May 31, 1920	27. 5	3. 7				
Constants computed from speci-men means.	_____	_____	26. 8	1. 18	19. 0	. 95	3. 85	[2] 1. 41

[1] Based on 200 spores.   [2] Geometric means of the above ratios.

METHODS

SECTIONING, STAINING, AND MOUNTING

The killing, sectioning, staining, and mounting methods described in the earlier paper on *Cronartium ribicola* (*1*) were followed in preparing slides for the study of the mycelium in the bark and wood, and the young aecia. The mature peridia were sectioned for the measurement study and for observations on comparative morphology either with hand razors or on an ether freezing microtome. The use of two sections of a Lima bean for holding the peridia, suggested by N. A. Cobb, made the sectioning process comparatively easy in spite of the brittle character of the objects. The hardness of the Lima bean was adjusted by soaking and redrying until it was just right for the job.

Aeciospores were shaken out of the aecia (not scraped out) or picked up on a knife blade from the bottom of the packet containing the specimen, and mounted in the same glycerin and glycerin-jelly media that were used in the study of the urediniospores (*3*).

Fragments and sections of the peridia were mounted in the same way.

It will be noted that the method of getting the aeciospores into the mounts assured both mature spores and a fairly good sampling of the spores from any particular specimen. Each mount made by shaking the spores out of the aecia into the mounting media presumably contained spores from several aecia; and each mount made from the mass of spores which were lying loose in the herbarium packet presumably contained a mixture of spores from all the opened aecia on the specimen.

MEASURING

The great majority of the spores were measured by means of a projection apparatus (*2*). The images of the spores, at a magnification of 1,000 diameters, were thrown on a white field; and those images which fell within a 4-inch circle in the center of this field were measured to the nearest millimeter with a white-face millimeter scale.

In all of the measurement work the mount was moved across the field of vision systematically by means of a mechanical stage. One hundred spores were measured from each of the specimens of each species. The thickness of the thickest visible part of the side spore wall was measured on 100 spores of each of 19 specimens of *Cronartium ribicola* and 14 specimens of *C. occidentale*.

The tubercles on the aeciospores were measured with a filar micrometer.

Measurements of the peridial cells, also made with a filar micrometer, were limited to the cells of the outer layer of the peridium. The walls measured were the outer walls of these same cells. The measurement results are presented in Tables 1 to 6.

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TABLE 2.—*Biometric data on the tubercles on the aeciospores of Cronartium ribicola and C. occidentale, based on 100 tubercles from each specimen*

Host	Locality	Date collected	Length		Width		Ratio: Mean length, mean width
			Mean	Standard deviation	Mean	Standard deviation	
Cronartium ribicola:			μ		μ		
Pinus strobus_____	Dunraven, N. Y____	May 20, 1915	1.64	0.35	1.16	0.24	1.41
Do_____	Exeter, N. H_____	May 24, 1917	1.56	.25	1.15	.22	1.35
Do_____	Kingston, Mass_____	Mar. 22, 1918	1.52	.28	1.10	.22	1.38
Do_____	Block Island, R. I___	May —, 1920	1.47	.28	1.06	.18	1.38
Do_____	Topsfield, Mass_____	Mar. —, 1921	1.39	.26	1.04	.17	1.33
Pinus monticola_____	North Vancouver, B. C.	June —, 1922	1.51	.32	1.08	.21	1.39
Do_____	_____do_____	June 7, 1922	1.53	.45	1.11	.21	1.37
Constants computed from specimen means.			1.52	.071	1.10	.041	[1] 1.37
Cronartium occidentale:							
Pinus edulis_____	Bayfield, Colo_____	May 19, 1918	2.26	.53	1.31	.24	1.72
Do_____	Mancos, Colo_____	June 29, 1918	2.30	.69	1.36	.29	1.69
Pinus monophylla___	Minden, Nev_____	May 22, 1920	2.21	.54	1.33	.26	1.66
Do_____	_____do_____	May —, 1920	2.26	.83	1.34	.29	1.68
Do_____	Carters Station, Nev.	June —, 1921	2.40	.62	1.32	.27	1.81
Constants computed from specimen means			2.29	.063	1.33	.017	[1] 1.71

[1] Geometric means of the above ratios.

TABLE 3.—*Biometric data on the outer layer of peridial cells in the aecia of Cronartium ribicola and C. occidentale, based on 100 cells from each specimen*

Host	Locality	Date collected	Length		Width		Wall		Ratio: Mean length, mean width
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Cronartium ribicola:			μ		μ		μ		
Pinus strobus___			42.7	6.0	31.5	7.3	6.16	1.24	1.35
Do_____	Kittery Point, Me.	May —, 1917	42.8	6.6	29.8	5.1	7.25	1.64	1.43
Do_____			44.1	7.2	29.4	4.5	7.45	1.64	1.50
Do_____	Kittery Point, Me.	May —, 1917	38.8	8.4	26.5	4.5	6.19	1.17	1.46
Do_____	_____do_____		42.8	6.4	28.7	4.5	6.82	1.10	1.49
Pinus monticola.	North Vancouver, B. C.	June 7, 1922	38.4	6.6	27.2	5.9	6.52	_____	1.41
Do_____	Vancouver, B. C__	June 9, 1922	43.7	6.7	30.4	5.7	7.74	_____	1.43
Do_____	North Vancouver, B. C.	June, —, 1922	38.2	6.9	24.3	5.1	9.12	_____	1.57
Constants computed from specimen means.			41.4	2.35	28.5	2.19	7.16	.919	[1] 1.45
Cronartium occidentale:									
Pinus edulis_____	Bayfield, Colo____	May 19, 1918	27.0	3.4	20.6	3.6	4.48	.81	1.31
Do_____	_____do_____	_____do_____	25.5	3.5	18.0	3.7	4.14	.90	1.41
Pinus monophylla.	Bridgeport, Calif__	May 31, 1920	28.7	4.9	18.7	2.9	5.00	.88	1.53
Do_____	_____do_____	_____do_____	27.8	3.7	18.6	3.5	4.95	1.02	1.49
Do_____	_____do_____	_____do_____	28.1	4.8	19.6	2.8	4.84	.82	1.43
Constants computed from specimen means.			27.4	1.10	19.1	.91	4.68	.326	[1] 1.43

[1] Geometric means of the above ratios.

TABLE 4.—*Biometric data on the "concomitant" cells in the aecia of Cronartium occidentale, based on 100 cells from each specimen*

Host	Locality	Date collected	Length		Width		Mean wall thickness	Ratio: Mean length mean width
			Mean	Standard deviation	Mean	Standard deviation		
			μ		μ		μ	
Pinus edulis_____	Bayfield, Colo____	June 19, 1918	21.8	3.0	17.2	2.5	4.64	1.26
Pinus monophylla_____	Bridgeport, Calif__	May 31, 1920	21.9	2.3	18.5	2.1	4.92	1.18
Do_____	____do_____	_____do_____	21.5	2.4	18.0	2.4	4 35	1.19
Do_____	____do_____	_____do_____	22.4	2.5	17.8	2.0	4.56	1.25
Do_____	Minden, Nev_____	June —, 1920	22.0	2.5	18.0	2.4	4.54	1.22
Constants computed from specimen means.	_____	_____	21.9	.29	17.9	.42	4 60	[1] 1.22

[1] Geometric mean of above ratios.

TABLE 5.—*Biometric constants for Cronartium ribicola and C. occidentale, computed from the populations of specimen means*

Dimensions measured	Cronartium ribicola					Cronartium occidentale					Difference between the two fungi			
	Number of specimens	Mean	Probable error of the mean	Probable deviation of a single specimen	Coefficient of variability	Number of specimens	Mean	Probable error of the mean	Probable deviation of a single specimen	Coefficient of variability	Difference between means	Probable error of the difference	Ratio: Difference, error	Diagnostic division point
Aeciospores.														
Length_____	25	24.2	0.15	0.76	4.6	21	26.8	0.18	0.81	4.4	2.6	0.23	11.3	25.5
Width_____	25	18.3	.16	.79	6.2	20	19.0	.15	.65	5.0	.7	.21	3.8	18.7
Wall _____	19	3.41	.081	.353	14.9	14	3.85	.074	.279	10.4	.440	.109	4.0	3.66
Length_____	} 25	[1] 1.32	.010	.053	5.8	20	[1] 1.41	.011	.050	5.1	.09	.015	5.3	1.365
Width_____														
Aeciospore tubercles:														
Length_____	7	1.52	.019	.051	4.7	5	2.29	.022	.048	2.8	.77	.029	26.6	1.92
Width_____	7	1 10	.011	.029	3.7	5	1.33	.006	.013	1.3	.23	.012	19.3	1.26
Peridial cells:														
Length_____	8	41.4	60	1.70	5.7	5	27.4	.37	.83	4 0	14.0	.70	20.0	31.9
Width_____	8	28.5	.56	1.58	7.7	5	19.1	.31	.68	4.8	9.4	.64	14.7	21.9
Wall _____	8	7.16	.233	.661	12.8	5	4 68	.110	.246	7.0	2.48	.257	9.6	5.33

[1] Geometric means.

TABLE 6.—*Summary of measurements of aeciospores of Cronartium ribicola and C. occidentale from different hosts*

Host	Number of specimens	Length	Width	Wall thickness
Cronartium ribicola:		μ	μ	μ
On Pinus strobus_____	22	24.0±0.15	18.1±0.15	[1] 3.24±0.06
On Pinus monticola_____	3	[2] 25.5± .41	[2] 19.8± .41	[2] 4.32± .14
Differences_____	_____	1.5± .44	1.7± .44	1.08± .15
Cronartium occidentale:				
On Pinus monophylla_____	12	27.2± .16	19.0	3.95
On Pinus edulis_____	8	26.3± .37	19.1	3.61
Differences_____	_____	.9± .40	.1	.34

[1] Based on 16 specimens only.
[2] Probable errors for these 3-specimen means are not computed from the population of 3 specimens. They are assumed to be the same percentage of the means as would be expected for 3-specimen means from *P. strobus.*

GENERAL OBSERVATIONS

There are certain differences in the habit of *Cronartium ribicola* and *C. occidentale* which are clearly evident to anyone who has had the opportunity to examine representative specimens or to observe the fungi in the field. For example, the pycnia of *C. ribicola* on *Pinus strobus* (*1, pl. 48, fig. B; 7, pl. II, fig. 2*), though occasionally confluent, are usually discrete and conspicuous; whereas the pycnia of *C. occidentale* on *P. monophylla* are made up of broad confluent groups of spore-bearing cells hidden beneath the overlying grayish outer bark.

The typical aecia of *Cronartium ribicola*, as they appear in the smooth soft bark of *Pinus strobus* (*1, pl. 48, fig. B; 5, pl. 55*), are usually separate sori, although occasionally they run together. The peridia, puffed out by the growing aeciospore chains, protrude through the bark—conspicuous bits of evidence that the host is infected. The aecia of *C. occidentale*, particularly on *P. edulis*, are broad confluent spore-producing layers which are more or less completely hidden under the hard bark—the spores massed at a crack in the bark being the only indication of infection. The area of the spore-bearing surface can not be determined until the overlying bark is removed. On young twigs of *P. monophylla*, however, the aecia are small, the peridia protrude, and the general appearance of the infected branch reminds one very strongly of branches of *P. strobus* infected with *C. ribicola*.

Hedgcock, Bethel, and Hunt (*5, p. 414*) are of the opinion that the difference in morphology is not due to the physical difference in the bark of the hosts. In the case of *Cronartium ribicola*, at least, the aecium which develops under the horny, resin-infiltrated bark of the canker area does not expand radially or in any way take on the character of the aecium of *C. occidentale* as it is commonly found under the hard bark of *Pinus edulis*. Aecia on the roots of *P. strobus* appear to be normal in size, shape, and structure (*1, p. 648*). There seems to be no way of ascertaining what form *C. ribicola* would take in the bark of *P. edulis*, or what form *C. occidentale* would take in the bark of *P. strobus*, unless it proves possible to grow the fungi on the hosts indicated. In the face of lack of evidence to the contrary, one is forced to conclude that the difference in habit of the aecia is real and specific. One must also conclude, however, that the type of aecium on the young branches of *P. monophylla* is totally unlike the confluent type on *P. edulis*. The former might be taken for *C. ribicola*, but the latter never. This fact and the data in Table 6 suggest that there may be a varietal difference between *C. occidentale* on *P. edulis* and *C. occidentale* on *P. monophylla*. The question of gradations between the type on the young branches of *P. monophylla* and the type on *P. edulis* must be passed over for the present.

MORPHOLOGIC AND BIOMETRIC COMPARISON

MYCELIAL CHARACTERS

The difference in the character of the bark of *Pinus strobus* and *P. edulis* naturally produces a difference in the appearance of sections of infected tissue. The hyphae and haustoria of the two rusts vary little in habit. The mean diameter of the hyphae in the wood for

both rust species, based on a total of 100 measurements made on six different specimens of each, is 4.9 μ. The walls of the same set of hyphae have a mean thickness of approximately 1 micron. Comparative measurements on the haustoria of the two forms—made on the largest haustoria, and therefore definitely selective—confirmed an impression gained from examination of hundreds of slides that the haustoria of *Cronartium ribicola* are longer than those of *C. occidentale*. The means of 100 measurements (*C. ribicola*, five specimens on *P. strobus; C. occidentale*, four specimens on *P. monophylla*) are as follows:

Haustoria of *C. ribicola*_____ 29.2μ long by 5.4μ in diameter, with a wall 1.1μ thick.
Haustoria of *C. occidentale*_____ 25.8μ long by 5.4μ in diameter, with a wall 1.1μ thick.

It is evident that difference in size, as far as these few specimens are concerned, exists only in the length of the haustoria. The difference is six and five-tenths times its probable error. The number of specimens sampled was so small, however, that positive conclusions are not justified. The character of the haustoria may be influenced by the difference in host.

COMPARATIVE MORPHOLOGY OF THE AECIOSPORES OF THE TWO SPECIES

The aeciospores of the two species appear to be very similar under medium power lenses. Under higher powers they are distinctly different. Both species produce characteristic Peridermium spores—obovoid to ellipsoid in shape, with a wall partly smooth and partly coarsely verrucose, as illustrated in Figure 1. Three points of difference in morphology stand out fairly clearly when the spores are compared, namely: The aeciospores of *C. ribicola* (fig. 1, A to G) are slightly smaller than those of *C. occidentale* (fig. 1, H to N); their outline is somewhat more regular; and their tubercles are more regular in shape and more evenly distributed. These differences have been taken into consideration in drawing the figures lettered O and P in Figure 1, which represent mean aeciospores of *C. ribicola* and *C. occidentale*, respectively, constructed on the basis of the mean size figures given in Tables 1 and 2.

One other difference which can not be illustrated in a drawing is a difference in light refraction at the edge of the spore. The wall and tubercles of *Cronartium occidentale* are more refractive than those of *C. ribicola*; and the difference is great enough to enable one familiar with the spores to pick out those of one species from those of the other when both forms are mounted under one cover. The greater refraction in *C. occidentale* appears to be due to the more irregular shape and distribution of the tubercles.

BIOMETRIC COMPARISON OF THE AECIOSPORES

The values given in this and the following sections will be found to differ only slightly from those published in the preliminary report (*4*) on part of the biometric study. The data are summarized in Table 1 and represented graphically in Figure 2. The mean wall thickness refers to the mean of measurements made for each spore at the thickest visible part of the side wall.

The aeciospores reach almost mature size soon after the division of the aeciospore initial into the aeciospore and intercalary cell. For instance, if the spores be numbered from the base of the aecio-
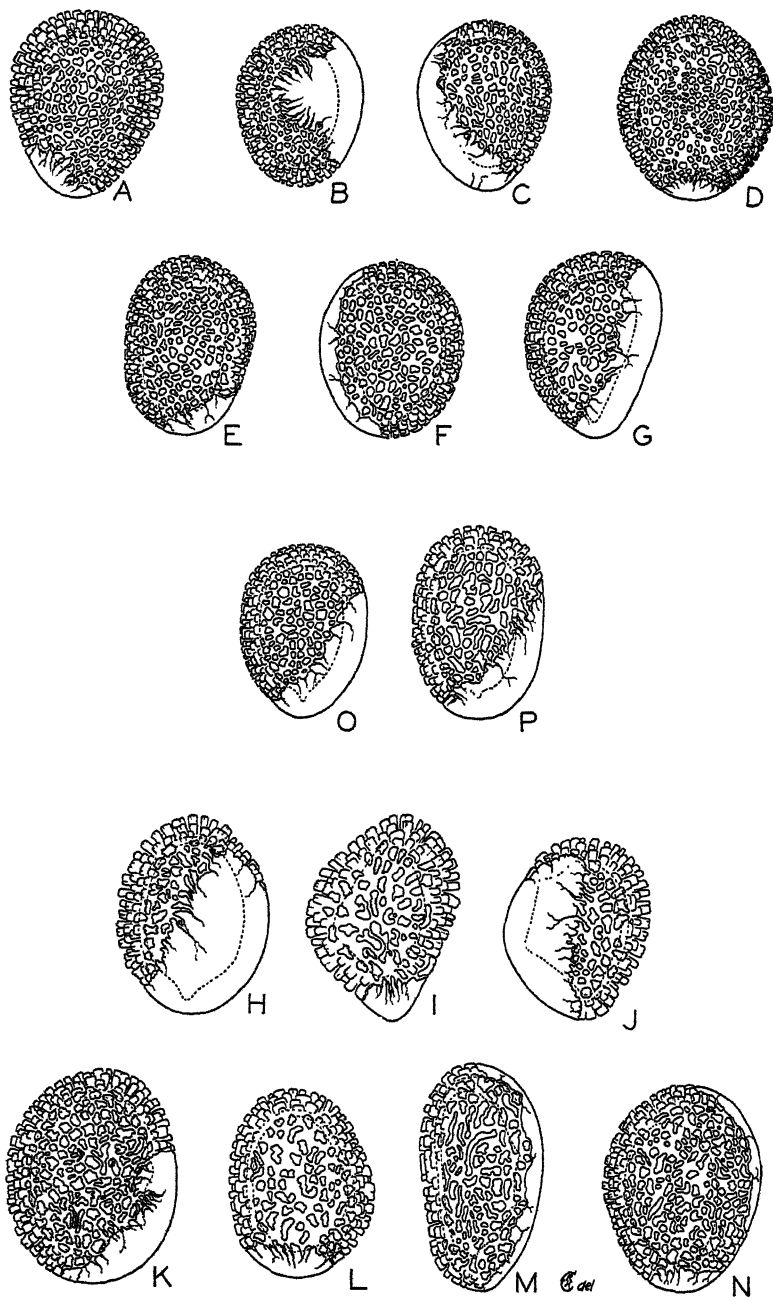
Fig. 1.—A to G, aeciospores of *Cronartium ribicola*, × 900; H to N, aeciospores of *C. occidentale*, × 960; O and P, mean aeciospores of *C ribicola* and *C. occidentale*, respectively, based on the mean size and shape data given in Tables 1 and 2

spore chain, No. 1 being the youngest, it will be found that the spores in No. 2 position are nearly full size in length, and that those in No. 3 and No. 4 positions are very close to the mean size figures of Table 1, though the walls are still decidedly thinner than in fully matured spores. The following data, from measurements made with a filar
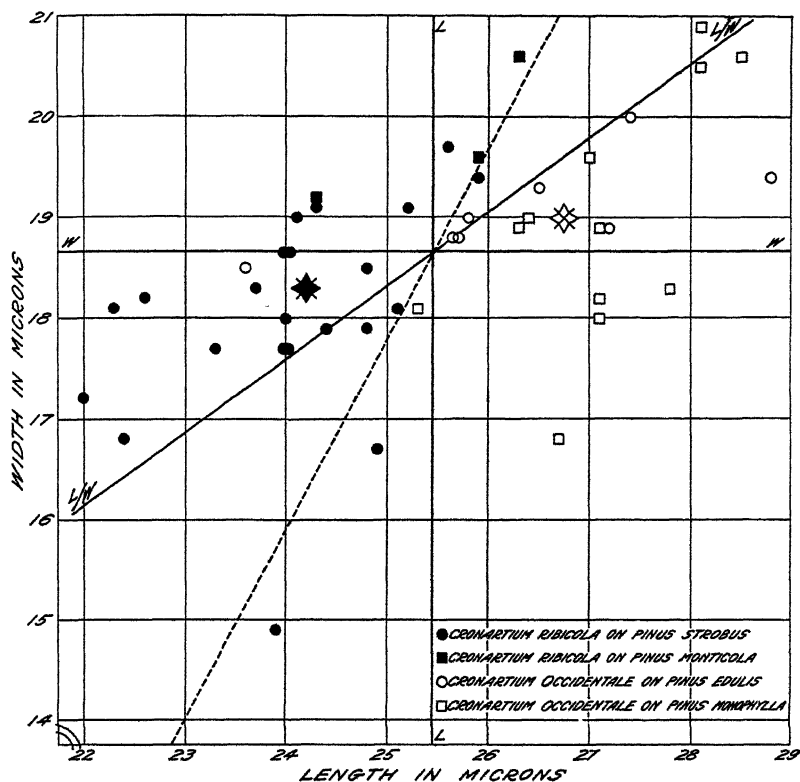
Fig. 2.—*Cronartium ribicola* and *C. occidentale*. Graphic methods for separating two species according to length and width of spores. The points are based on 100-spore means, and each represents a different specimen. The stars indicate the means for the two species. The solid lines are the most probable diagnostic separation lines determined by computation; the line marked W is for width, the line L is for length, and the line marked L/W is for the ratio of length to width. The broken line is located empirically as being apparently the best straight line for separating the two species

micrometer on stained sections mounted in balsam, support the conclusion:

Cronartium ribicola (two specimens, three slides from each):
    Second spore in chain.
        Means from 26 spores, $23.5 \times 11.7\mu$, wall 1.9μ.
    Third spore in chain.
        Means from 20 spores, $24.1 \times 15.0\mu$, wall 1.9μ.
    Fourth spore in chain.
        Means from 4 spores, $23.6 \times 13.4\mu$, wall 1.9μ.
Cronartium occidentale (three specimens, one slide from each):
    Second spore in chain.
        Means from 26 spores, $26.7 \times 16.6\mu$, wall 2.7μ.
    Third spore in chain.
        Means from 20 spores, $28.3 \times 17.3\mu$, wall 2.8μ.
    Fourth spore in chain.
        Means from 4 spores, $27.3 \times 17.1\mu$, wall 2.7μ.

Measurements made on stained spores mounted in balsam are, of course, not directly comparable with the measurement figures in Table 1; and the number of measurements reported is small; but the means are at least indicative. The relatively larger size and thicker wall of the aeciospores of *Cronartium occidentale* are apparently characteristic even before the spores are mature.

Hedgcock, Bethel, and Hunt (*5*) have already called attention to the size of the tubercles on the aeciospores of *Cronartium occidentale* in their description of the species. Morphologic differences in such minute things as tubercles are easily overlooked when one is studying rust spores in a routine way under the medium power of the microscope; yet the recognition of these differences is essential to any true diagnostic description. Data on the size and shape of the aeciospore tubercles are given in Table 2. Length and width in the case of the tubercles mean the long and short dimension, as nearly as they can be measured, of the end views of the tubercles as they appear in Figure 1. The means in the summaries of Table 2 were obtained from measurements of 500 tubercles for *C. occidentale* and 700 for *C. ribicola*, approximately 5 of the most clearly visible tubercles having been measured on each of 20 spores in the case of each of the specimens. The greater length and greater irregularity in shape and distribution of the tubercles in *C. occidentale* is clearly evident in the spores shown in Figure 1.

COMPARATIVE MORPHOLOGY OF THE PERIDA OF THE TWO SPECIES

General observations have indicated a decided difference in the peridia of the two forms. The peridium of *Cronartium ribicola* has been described as thick and persistent in contrast to the inconspicuous, "thin, evanescent" (*5, p. 414*) peridium of *C. occidentale*. Colley's drawings of a section of the peridium of *C. ribicola* (*1, pl. 56, fig. B*) show it as three to four cells thick. Vertical sections through the aecium (*1, pl. 50, fig. B*) show that this multilayered cover extends all over the aecium, and that before the rupture of the bark by the force of the growth of the aeciospore chains the peridium forms a very definite layer between the tips of the young spore chains and the overlying bark and "buffer" tissue. The peridium of *C. occidentale*, whether it remains closely pressed against the overlying bark tissue of *Pinus edulis*, or whether it protrudes through the bark as in the case of infections on young branches of *P. monophylla*, generally appears to be but one cell in thickness. Occasionally a few cells are found in the position of a second, or inner, layer.

The thickness of the cell wall decreases in each layer of cells in the peridium of *Cronartium ribicola* (fig. 3, A); and as a rule the outer wall is thicker than the inner wall of any given cell. The wall thickness remains practically constant for any given cell in *C. occidentale*. Under proper illumination the thick walls are beautifully striated (fig. 3, A and B). The cells retain some of their contents even after the peridia become quite dry. Only rarely are the inner walls of the outer layer of cells marked with tubercles in *C. occidentale*; whereas all the cells are sculptured in the case of *C. ribicola*. The sections shown in the drawings are typical of a large number examined, taken from numerous specimens and localities.
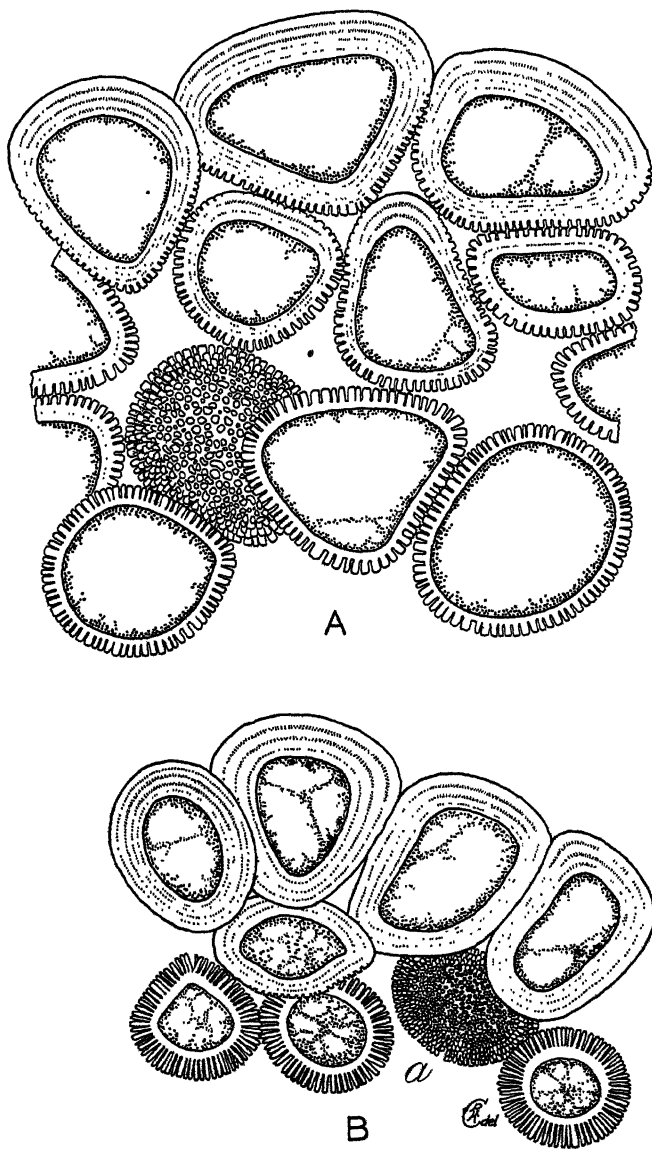
FIG. 3.—A detailed sectional view of small segment of roof of peridium: A, *Cronartium ribicola* on *Pinus strobus*; B, *C. occidentale*; a, "concomitant" cells. × 1,000

A comparison of the illustrations in Figures 4 and 5—sections of the peridia of *Cronartium ribicola* and *C. occidentale*, respectively— brings out the major differences between the two much more clearly than could any description. Particular attention is directed to the cells shown in Figure 3,B,*a*. These "concomitant" cells form a layer in connection with the peridium of *C. occidentale* which may correspond to one of the inner layers of the peridium in *C. ribicola;* but they resemble neither the peridial cells above them nor the spores below. The concomitant cells of *C. occidentale* are so highly refractive that they stand out very clearly when a fragment of the peridium is mounted with the under side toward the objective; and, as a matter of fact, they alone would probably serve to identify the species. If fragments of peridia of *C. ribicola* and *C. occidentale* are mounted side by side under the same cover the two appear to be absolutely distinct. Further work
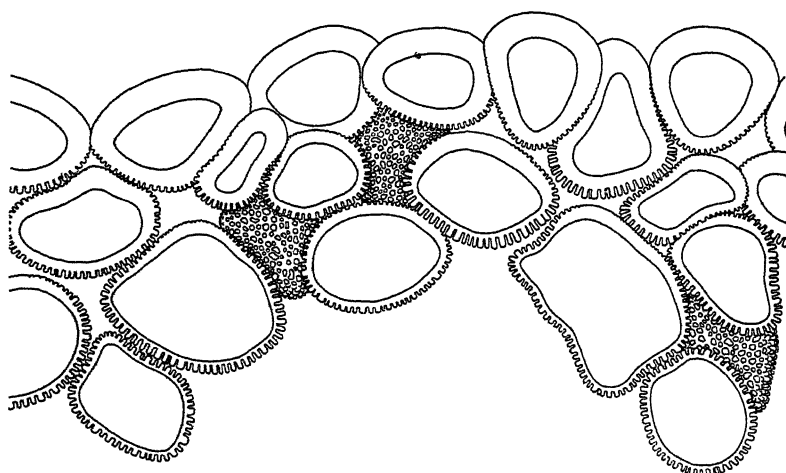
Fig. 4.—Outline drawing of a section of the peridium of *Cronartium ribicola* on *Pinus strobus*, cut near the central or dome region. × 585

will probably show peridial characters to be as important for the Peridermium group as Kern (*6*) has found them to be for Gymnosporangium.

BIOMETRIC COMPARISON OF THE PERIDIAL CELLS

Measuring the size of the peridial cells in surface view was found to be so difficult and the results so generally unsatisfactory that it was decided to use sections of the peridia for the biometric study, and to measure only the long and short dimensions of the outer layer of cells of the peridium, and the thickness of the outer wall of these same cells. In general the cells measured were in the roof rather than in the side walls of the peridia. The results are given in Table 3.

Biometric data on the size of the concomitant cells are given in Table 4. If these figures be compared with the figures for the size of the peridial cells and with the figures for the size of the aeciospores, it becomes evident that the concomitant cells are smaller than either. It is difficult to determine the size of the tubercles on the concomitant cells on account of their small size in end view, and because they are frequently compacted into irregular groups. They average something

less than 1μ in diameter in surface view. The obvious differences between these tubercles and the aeciospore tubercles are illustrated in Figures 1 and 3.

SIGNIFICANCE OF BIOMETRIC DIFFERENCES

The qualitative differences already described show rather clearly that the two rusts are different. To determine whether their morphologic differences are sufficient to warrant giving both of them specific rank, it is necessary to examine the significance of the differences which they exhibit in as many characters as possible.

The differences between the two rusts in the size of the outer layer of peridial cells are so large and consistent as to leave no doubt as to their reality. While the measurement data were obtained only from eight specimens for one species and five specimens for the other, two host species and at least two widely separated localities were represented for each fungus. The 100-cell length means from the different specimens all lie between 25 and 29μ for *Cronartium occidentale*
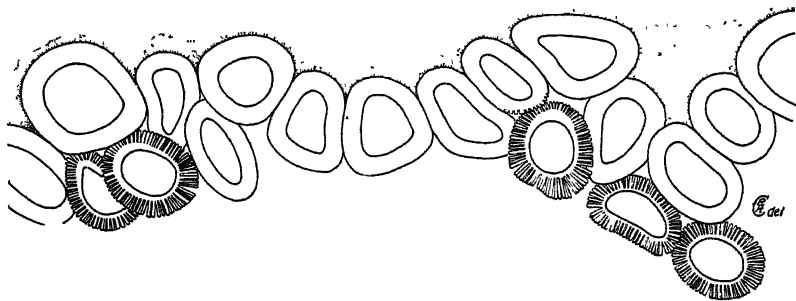
FIG. 5.—Outline drawing of a section of the peridium of *Cronartium occidentale*, cut near the central or dome region. × 585

and between 38 and 45μ for *C. ribicola*. The differences between the two species in the width and wall thickness of the peridial cells are of nearly the same order of decisiveness.

A glance at Table 2 shows decided differences in the dimensions of the surface view of the tubercles. For the seven specimens of *Cronartium ribicola*, from two hosts and two entirely different regions, the 100-measurement length means are all below 1.65μ; while for the five specimens of the other species, from two hosts and four localities, the length means are all above 2.2μ. The difference in tubercle width is less striking but apparently significant. It is evident that the populations which furnished these measurements of tubercles and peridial cells are quite distinct. Emphatic confirmation of the conclusion reached by inspection is furnished by the probability computations based on the measurements of these units. (See Table 5.)

In the case of the aeciospores themselves, the difference between the species is less obvious. The fact that the dimensions for different specimens overlap is best shown in Figure 2. The differences between the aeciospore species means are relatively much smaller than the differences which were found in the case of the tubercles and peridial cells. These points are evident after an examination of the probability constants shown in Table 5. The difference in

spore length between the two species means, though only 10 per cent, is more than 11 times its probable error, and on the face of things is undoubtedly significant. In spore width, wall thickness, and ratio $\frac{\text{mean length}}{\text{mean width}}$ the two species show less decisive differences with respect to their probable errors. In any case differences in these other characters do not furnish entirely independent support of the species difference, because all three factors are correlated with length. For *Cronartium ribicola* aeciospore lengths the correlation coefficient with width is 0.59 ± 0.09, and with wall thickness 0.61 ± 0.10. For *C. occidentale* lengths the correlation with width is 0.54 ± 0.11, with wall thickness 0.53 ± 0.13, and with the ratio of length to width 0.43 ± 0.12.

There remains the question as to whether the significant difference between the two populations sampled as to aeciospore lengths is really genetic, or simply due to differences in climate or substratum. These are questions on which the above probability figures give no information. The possibility that the larger spores of *Cronartium occidentale* are due to climatic influence can be practically ignored for the reason that it grows in a much drier climate than that in which the *C. ribicola* collections were made; and it is the general observation that the effect of drought, if any, is to decrease spore size. It is less easy to dispose of the possibility that the difference is due to substratum. So far as one can judge, if there had been more *C. ribicola* measurements from *Pinus monticola* the difference in average spore length between the two rust species would have been less. In the same way *C. occidentale* produced spores on *P. edulis* which differed less from *C. ribicola* than did those on *P. monophylla*. The aeciospore measurements are summarized separately by hosts in Table 6.

In the case of *Cronartium ribicola* it is impossible to reach definite conclusions as to the meaning of the difference between the spores from the two hosts, though all three of the specimens from *Pinus monticola* have larger spores than the mean of the spores from *P. strobus*, and the best probability computations possible under the circumstances indicate the difference to be significant. The specimens of *C. occidentale* were more evenly divided between its two hosts. The difference between the mean length of the aeciospores from *P. monophylla* and from *P. edulis* is scarcely more than twice its probable error. If the spores produced on *P. monophylla* are regularly larger, it is evident that measurements must be made from a larger number of specimens to establish the fact. Within each fungus species the difference between spore lengths from different hosts is small as compared with the difference in spore length between the species. The evidence is strong, though not absolutely conclusive, that irrespective of hosts, the spores of *C. occidentale* are longer than those of *C. ribicola*. To make it conclusive, it would be necessary to have measurements of each fungus from several congenial hosts. The ideal would be to have for comparison measurements of both fungi on a common congenial host, but it is very improbable that such a host exists. The wide spores and thick walls produced on *P. monticola* may indicate substratum effect, but perhaps they are the result of variation in sampling, of the moist atmosphere of Vancouver, or of an especially large-spored tendency in the strain of *C. ribicola* in

the single nursery-stock shipment which was the source of the Vancouver rust epidemic. Substratum effect is the most likely explanation, as the single specimen on *P. strobus* from Vancouver was decidedly below average in all the qualities in which the specimens from *P. monticola* were so high. There was striking agreement between the three *P. monticola* specimens in wall thickness; the possibility of poor sampling of the spores from the specimens measured was decreased by measuring 200 each from two of the specimens instead of the usual 100.

It will be noted that the probability constants in Tables 5 and 6 are computed from the population of specimen means, and not from the measurements of the individual spores. The latter procedure has been the one usually followed in the application of probability computations to spore measurements. It is theoretically at once evident that such a practice is unsound, for the reason that a sample composed of numerous spores from each of several specimens is not a simple sample such as one must choose for ideal probability computations. It is further evident that the number of individuals included in the sample is indicated not by the number of spores, but by the number of specimens. To substitute the number of spores for N in the denominator of the probable error formula will therefore give too low a value for the error. Empirical evidence of the lack of meaning of the probable error computed in the usual way from the individual spore measurements can be readily obtained from the data in the present paper. The error of the mean of the first 2,000 measurements of aeciospore lengths which were made for *C. ribicola* is 0.038μ. If this were valid for the species, the probable deviation of a mean of 100 measurements from the species mean should be $(2,000/100)^{1/2} \times 0.038\mu$ or 0.17μ. As a matter of fact, of the 20 specimen means, 17 show a larger deviation. Proceeding in the same way, it is found that 18 of the first 20 specimen means of *C. occidentale* differ from the species mean by more than the probable deviation (for this species 0.20μ). In fact, 11 of the 20 specimen means of the first fungus and 11 of the 20 means of the second actually differ from the species mean by more than three times the probable deviation figured from the 2,000-spore basis. In normal, simple populations only two such deviations would be expected in 47 submeans. It is undoubtedly true that means of 100 measurements taken at random from 20 or 25 different specimens would not so radically exceed the probable deviation; but it nevertheless appears that the only conservative procedure in calculating probability constants applicable to such a group as a species is to compute probable deviations or errors from a population of specimen means rather than from a population of individual measurements. In the present case the probable errors so computed are nearly four times as large as those obtained from the individual measurements, and the deviations of the specimen means quite closely follow the expectations based on these larger errors.

THE USE OF THE BIOMETRIC DATA IN THE IDENTIFICATION OF UNKNOWN SPECIMENS

Because the aecial stages of the two rusts have in the past inhabited different geographic regions and different hosts, there has been so far no need for microscopic identification of specimens. With the spread of *Cronartium ribicola* in the western part of the United States

the geographic criterion will no longer be valid, and it is entirely possible that either rust may be found capable of infecting one of the aecial hosts on which the other occurs. To avoid possible future confusion in the campaign which is being made for the control of *C. ribicola* in the West, it is desirable that there be available as many diagnostic criteria as possible. This is particularly true since some of the criteria may be changed as a result of change of host, and others, such as those dependent on the peridia, may be unavailable in weathered specimens. The size of the tubercles on the aeciospores is probably the character which may be depended on as most reliable and always available for examination. However, its determination requires very high magnification, and the aeciospores themselves will ordinarily be the character first measured. Because the differences between the two species in the dimensions of the aeciospores are small, the most probable diagnostic division point between them has been determined. Variability and probability constants based on the populations of specimen means—not of individual measurements—were employed in the determination. Obviously, if two species are equally variable the division point will be halfway between them; but if one species is more variable than the other, the division point should be farther from it and nearer the less variable species. The distance of the division point from each species mean should be proportional to the variability of the species. The division point located by the method described in the following paragraph is believed to have a higher probability of correctness than that determined by the inspection method used in earlier work with the uredinial stages of the two fungi (*3*).

The point is to be so located that the probability of a mean of measurements from a specimen of the smaller spored species being *above* the point would be equal to the probability of a specimen mean from the larger spored species being *below* the point. If A is the mean of the population of means for the larger spored species, B the mean for the smaller spored, σ_a the standard deviation of the A population, σ_b the standard deviation of the B population, and X the diagnostic division point, the conditions of probability referred to may be expressed by the formula

$$\frac{A-X}{\sigma_a} = \frac{X-B}{\sigma_b}$$

Solving for X[4]:

$$\sigma_b A - \sigma_b X = \sigma_a X - \sigma_a B$$

$$X(\sigma_a + \sigma_b) = \sigma_b A + \sigma_a B$$

$$X = \frac{\sigma_b A + \sigma_a B}{\sigma_a + \sigma_b}$$

Substituting the values for aeciospore length recorded in Table 1

$$X = \frac{(1.11 \times 26.8) + (1.18 \times 24.2)}{1.18 + 1.11}$$

$$= 25.46$$

---

[4] The writers are indebted to T. R. C. Wilson for an improvement in the formula which they originally used for this purpose.

The probable deviation or error of a single specimen can be used in the formula in place of the standard deviation without affecting the result. The average deviation may also be used without serious loss of accuracy.

The diagnostic division point for aeciospore length, 25.46, is distant from the means 26.8 and 24.2 by approximately 1.66 times the probable deviations of single specimen 100-spore means, 0.81 and 0.76, respectively, for the two species. A deviation 1.66 times the probable deviation would be expected approximately 26 times in 100 trials, and only 13 times in the direction of the diagnostic division point. Assuming that the distribution approaches normal and that the specimens are in each case a good sample of their species, the chances are that about 13 per cent of all specimens diagnosed on the basis of the length mean would be diagnosed incorrectly.

The diagnostic division points obtained in this way for the other characters measured are shown in Table 5. For the ratios of length to.width, variability and probability computations based on the logs of the specimen ratios would be somewhat preferable; but the variability in the ratios is small enough so that the differences in the results would be negligible. From the probability data, it appears that the chances of wrong diagnosis are approximately 36 per cent on the basis of width, 32 per cent on the basis of wall thickness, and 30 per cent on the basis of the $\frac{\text{mean length}}{\text{mean width}}$ ratio. The possibility of wrong diagnosis from the dimensions of aeciospore tubercles and of the peridial cells would be negligible.

Of course it must be understood that the bases for all these probability computations are inadequate in the number of specimens represented to justify entire confidence in them. The reliability of the figures given is also somewhat lessened by the fact that the specimens of each species have been treated as simple samples of a homogeneous population, when it is entirely possible that additional data would have shown that the measurements of each fungus are significantly affected by differences in host species. The diagnostic division points and the probability figures simply are the best results that can be condensed out of the available data. The actual number of specimens of the material investigated that would have been wrongly determined by application of the diagnostic division points is 6 out of 46 for aeciospore length, 16 out of 45 for width, 12 out of 33 for wall thickness, and 9 out of 45 for ratio $\frac{\text{mean length}}{\text{mean width}}$. None of the specimens whose aeciospore markings and peridial cells were measured would have been diagnosed wrongly on the basis of the dimensions of these structures.

The length, width, and $\frac{\text{mean length}}{\text{mean width}}$ data from Table 1 are shown graphically in Figure 2. Each plotted point represents both the mean length and the mean width of 100 measurements; in other words, the mean size of 100 spores. For example, the solid dot at the intersection of loci 24.4 and 17.9 represents the length and width means of *Cronartium ribicola* from Dunraven, N. Y. (See Table 1.) The stars represent the species means. The vertical line at 25.46 represents the diagnostic division for length, the horizontal line at 18.68 represents the point for width, and the solid diagonal line

drawn to satisfy the equation, length $= 1.365$ width represents the division for $\dfrac{\text{mean length}}{\text{mean width}}$. The figure serves much better than a table to illustrate the way the mean sizes of the spores from different specimens vary from the species mean and with respect to the diagnostic division lines, and the extent to which the ranges of the two species overlap. For biometric diagnosis, length appears from every viewpoint to be the most important of the aeciospore dimension criteria so far discussed, and if one were called upon to make a diagnosis of a white pine or nut pine Peridermium after an examination of the spore dimensions alone the length criterion would naturally be applied first.

A SINGLE DIAGNOSTIC INDEX WHICH USES BOTH LENGTH AND WIDTH

It will be noted that the line on Figure 2 which represents the most probable separation of the two species on the basis of spore length is vertical, that for width is horizontal, and that for the ratio of length to width has its origin at 0 for both length and width. It is at once evident that the line which will best separate the two "swarms" of specimen means shown in the graph will rarely happen to be an exactly horizontal or vertical line, or one which starts at zero for both length and width. It is evident from the graph, and has been confirmed by the coefficients given on page 524, that length, width, and their ratio are so correlated within each swarm that the criteria are not independent, and there is little advantage in trying to employ them separately for diagnostic purposes. In such cases the writers recommend that the line which seems best to separate the two swarms be located by inspection. The broken diagonal line in Figure 2 was so located. For all points on the line, length is $15.5\mu + 0.553$ width. This value was found after the line had been located, by noting that it cut the zero width line at 15.5 on the length scale, and increased 0.553μ on the length scale for each 1μ increase on the width scale. Its significance is that if a specimen has spores whose average length is more than $15.5\mu + 0.553$ of their average width, it is considered as probably *Cronartium occidentale;* if the average length is less than this value, the specimen is probably *C. ribicola.* Of the 45 specimens for which both lengths and widths are available, it will be seen on the graph that 4 would be wrongly identified by this criterion. For this particular lot of data, the vertical diagnostic length line happens to separate the swarms nearly as well as any other line which can be drawn.

While the slope of this line has been decided by inspection, it has been pivoted on the intersection of the most probable division values for length and width, and is probably more accurate than if located entirely by inspection. Its slope could be determined mathematically by describing for each swarm a contour of the best fitting frequency surface for that swarm. The points at which the contours for the two swarms intersect would be loci of the most probable, or at least of a very probable, straight line of separation. It is difficult to imagine any situation in mycological work which would justify the amount of computation required by such a process.

Where the correlation between length and width is not rectilinear, or where the two swarms differ materially in shape or degree of spread

the best line of separation of two swarms will be a curve. The determination of the best curved line would be a still more difficult matter, and the advantage over a straight line, if any, would probably be slight.

GENERAL DISCUSSION

One of the populations which have been compared in the present paper has included Cronartium individuals occurring on a 5-needled pine in the northeastern part of the United States, together with a smaller population on this and another 5-needled pine in British Columbia. The other population has included Cronartium individuals which grow on two nut pines in the southwestern part of the United States. The two populations were at one time confused; they were classed as separate species, chiefly on the ground of differences in host preference. In an earlier paper one of the present writers has shown that there is a small quantitative morphologic difference between the two fungi in the urediniospores when grown under outdoor conditions, whether in their respective natural habitats, or as a result of inoculation on the same hosts and under the same conditions. The data in the present paper show that the aecial stages differ very strikingly in some morphologic characters, and in others display smaller differences which by biometric methods can be shown to be significant. The differences which are so large that biometric demonstration is not required are of course the most important ones from every standpoint. It has not been possible in the aecial-stage comparisons to eliminate the possible influence of different climate or host, but the evidence indicates that the major portion of the differences observed are genetic, rather than the effect of environment or substratum. Added to the differences which had previously been demonstrated (5), these aecial differences seem sufficient to end any question as to the independent specific rank of *C. occidentale*.

These morphologic differences in the aecial stage should also be helpful in situations which may require the determination of specimens from unidentified host bark, or where there is suspicion that one of the species has jumped to a host outside of its usual host range. It is well to point out, however, that unless methods strictly comparable to those used in this paper are employed, other investigators can not expect the means and diagnostic division points to hold good, and they will have to rely on the qualitative morphologic differences for diagnosis.

The following descriptions of the aeciospores and peridia of the two forms combine the morphologic and biometric data. The figures are the same as, or are based on, the averages of the populations of means shown in Table 1:

Cronartium ribicola Fischer—

Peridia thick, persistent; 3 to 5 cells thick; outer layer of cells in region near dome, sectional view, long and short dimensions (basis 100 measurements from each of 8 specimens) 41.4 by 28.5μ, standard range [5] of the 8 specimen means, 39–43.8 by 26.3–30.7μ; outer wall of cells in outer layer smooth 7.16μ thick, generally thicker than inner wall; inner wall marked with short

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[5] The lower and upper limits of the standard range differ from the mean by the amount of the standard deviation. Ordinarily about two-thirds of the specimens should have measurements within the standard range.

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tubercles; outer walls of cells in second and third layers sometimes smooth, sometimes minutely warted, inner walls marked with tubercles; walls of cells in the inner layers generally uniformly marked with tubercles; walls progressively thinner from the outer layer to the inner layer of the peridium; outer walls of cells in first two or three layers usually thicker than the inner walls.

Aeciospores obovoid to ellipsoid, generally smoothly curved in outline, (basis 100 spores from each of 25 specimens) 24.2 by 18.3μ, standard range of the means 23.1–25.3 by 17.2–19.4μ; ratio mean length divided by mean width, geometric mean of the values for the 25 specimens 1.32; wall (basis 100 spores from each of 19 specimens) 3.41μ; wall partly smooth and partly marked with tubercles, the smooth area fissured near junction with tubercles; tubercles fairly regular in outline in end view (basis 100 measurements from each of 7 specimens), 1.52 by 1.10μ, standard range of the means 1.45–1.59 by 1.06–1.14μ.

Cronartium occidentale Hedg. Bethel, and Hunt—

Peridia thin, evanescent; one cell, rarely two cells thick; outer layer of cells in region near dome, sectional view, long and short dimensions (basis 100 measurements from each of 5 specimens) 27.4 by 19.1μ, standard range of the means 26.3–28.5 by 18.2–20.0μ; outer wall of outer layer of peridial cells 4.68μ; outer wall of cells in outer layer smooth, inner wall generally smooth, occasionally marked with minute tubercles; "concomitant" cells just below outer layer of cells of peridium (basis 100 measurements from each of 5 specimens) 21.9 by 17.9μ; standard range of the means 21.6–22.19 by 17.5–18.3μ; wall of concomitant cells 4.60μ; tubercles minute in end view, averaging somewhat less than 1 by 1μ, often compacted into irregular clumps.

Aeciospores obovoid to ellipsoid, often somewhat irregular in outline (basis 100 spores from each of 20 specimens) 26.8[6] by 19μ; standard range of the means 25.6–28 by 18–20μ; ratio mean length divided by mean width, geometric means of the values for the 20 specimens, 1.41; wall (basis 100 spores from each of 14 specimens) 3.85μ; wall partly smooth and partly marked with tubercles, the smooth area fissured near junction with the tubercles; tubercles irregular in outline in end view (100 measurements from each of 5 specimens) 2.29 by 1.33μ; standard range of the means 2.23–2.35 by 1.31–1.35μ.

A comparison between Figure 2 of the present paper and Figure 4 of the urediniospore paper (*3*) reveals the fact that the urediniospores and the aeciospores of *Cronartium occidentale*, respectively, differ in size and shape from the urediniospores and aeciospores of *C. ribicola* in the same general way. Reduced to percentages based on mean spore size, the urediniospores of *C. ribicola* may be described as 93 per cent as long as those of *C. occidentale*, and the aeciospores of *C. ribicola* as 90 per cent as long as those of *C. occidentale*. Whether this same general relation holds for the teliospores and sporidia remains to be seen.

None of the measurement distributions encountered gave indications of real bimodality. Skewness was more commonly positive; no extreme tendency to skewness was seen.

In the case of two forms which are as close together in their host relationships and morphology as *Cronartium ribicola* and *C. occidentale*, extreme spore-size ranges are utterly useless for diagnostic purposes. The statement in an earlier paper (*1, p. 632*) that the mature aeciospores of *C. ribicola* measure "18 to 21 by 20 to 26μ" tells only part of the story. In the present study, for instance, the extreme ranges for *C. ribicola* and *C. occidentale* are 16–43 by 11–25μ and 19–40 by 9–28μ, respectively. In which species would spores described by the first range cited be placed? The critical case presented by these two Cronartium species serves excellently to illustrate the fact that averages are more useful than ranges in certain types of taxonomic work.

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[6] Length based on 21 specimen means.

SUMMARY

General observations have indicated marked differences in the habit of the aecia of *Cronartium ribicola* and *C. occidentale*. In the former the aecia are usually distinct sori with persistent peridia; but in the latter the aecia vary from distinct sori, e. g., on *Pinus monophylla*, to broad spore-bearing layers under the bark, e. g., as in infections on *Pinus edulis*, with thin inconspicuous peridia.

The aeciospores of *Cronartium occidentale* are slightly longer and wider than those of *C. ribicola;* they have a slightly thicker wall, a more irregular outline, and larger and more irregularly distributed tubercles.

The peridia of *Cronartium ribicola* are 3 to 5 cells thick, all the cells being marked with tubercles; the peridia of *C. occidentale*, on the other hand, are rarely more than 1 cell thick, and the cells in this one layer are rarely marked with tubercles. Certain cells, unlike the peridial cells or the aeciospores, are located just beneath the peridium of *C. occidentale*. These cells are given the name "concomitant cells."

Any unweathered specimen of *Cronartium occidentale* can be distinguished from any specimen of *C. ribicola* by any one of the following characters: Length, width, or shape of aeciospore tubercles; length or width of outer peridial cells; smoothness of outer peridial cells; and the contrast between concomitant cells and the cells above and below them. In aeciospore lengths, and in the gross characters of the sori, it is usually possible to distinguish between the two species, but in occasional specimens the two rusts overlap in these two characters.

The morphologic differences between *Cronartium ribicola* and *C. occidentale* seem quite sufficient to justify their standing as separate species.

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BURRKNOT FORMATIONS IN RELATION TO THE VASCULAR SYSTEM OF THE APPLE STEM [1]

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INTRODUCTION

Knight (8)[3] in 1809 mentioned the occurrence on the stems of certain varieties of apples of "rough excrescences, formed by congeries of points which would have become roots under favourable circumstances." Recently the writer (11, 12, 13) has called attention to the work of Knight and of others and has shown that many of our present commercial varieties of apples can be grown from cuttings containing burrknots, as Knight called these rough excrescences. The present paper deals with the anatomy of burrknot formation, a study undertaken in order to throw more light upon the physiological problem of the formation of adventitious roots on stems.

Kissa (7) in 1900 briefly described a case of "goiter gnarl" on *Malus chinensis* (*Malus spectabilis* Borkh.?), which was undoubtedly burrknot and which involved the production of adventitious roots. He showed that such roots arise as swellings at the outer edges of medullary rays, that growth in length of the tips is stopped by dryness, and that a lateral, basal branching takes place within the protecting cortex of the stem. Kissa made no reference to any definite location of these roots in relation to the primary vascular structure of the stem.

Borthwick (2) in 1905 published descriptions and photographs of adventitious roots in the apple and the maple, as well as in *Pyrus aucuparia*, *Ulmus campestris*, *Thuya gigantea*, and *Cupressus pisifera* (*Chamaecyparis pisifera*?). He shows that such roots arise at the outer edges of medullary rays, and that the cambium of the stem is connected with the cambium of the root, but he makes no mention of the relation of these roots to the primary vascular system.

Van der Lek (9) seems to have been the first to recognize the relation between the primary vascular system and the distribution of "root germs" on woody plants. Working with willow, poplar, and black currant, he found that most of the numerous root germs—he found 36 in one internode—arise in definite relation to the primary vascular system. He distinguishes between nodal roots—those which actually arise on the leaf traces, or branch traces themselves—and internodal roots—those which arise on the medullary rays. In some species the root germs are largely internodal, in others chiefly nodal.

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METHODS AND MATERIALS

The work here reported was done in the botanical laboratory of Johns Hopkins University, Baltimore, Md. For the study of root anatomy, material of the apple varieties Buckskin, Jersey Sweet, Rambo, Wagener, and Springdale was used. The material was removed from 25-year-old trees in the orchard at Arlington Experiment Farm, Rosslyn, Va., cut into ¾-inch lengths, and placed in Bouin's picric acid-formalin-alcohol killing fluid. The air was removed under vacuum to insure the penetration of the killing fluid. After 24 hours the material was washed in 40 per cent alcohol, which was changed several times during two or three days. The material was then kept in 60 per cent commercial hydrofluoric acid for two to four days, or until it was soft enough to cut easily with a knife. It was next washed in water until no hydrofluoric acid taste could be detected. The material was next run up through the alcohol. Since it was desired to show cell walls rather than cell contents, a more rapid transition was used than is desirable for cytological work. It was necessary to thoroughly remove all water by changing the absolute alcohol several times. The total time in the various alcohols was usually about five days.

The hydrofluoric-acid treatment necessary to soften 5 to 10 year-old wood made the material so brittle that it was necessary to embed it in celloidin. Plowman's method, as given by Chamberlain (*4*), was used. However, in order to determine the exact position of the root germs in relation to the leaf and branch traces, it was absolutely necessary to have serial sections. To get these a modification of Wilhelmi's (*14, p. 17*) method was used. After the celloidin-embedded material had been hardened in chloroform it was put into xylol (which was changed several times) and then embedded in paraffin. This made it possible to cut serial sections on a rotary microtome and to get sections of 8 to 10 microns in thickness of apple stems 10 years old. By putting the ribbons containing the sections on the slide and keeping them in place with mosquito netting, tied on with thread, it was possible to stain in series on the slide. Safranin and gentian violet proved the best stains. Some study was made of free-hand sections of fresh material. But for securing complete thin sections of wood the double embedded method was found to be necessary.

The structure of the primary vascular system was studied in new growth taken from the varieties listed above and also from the varieties Delicious and Tolman. Paraffin sections and free-hand sections were used. In the latter case the bundles were traced by haematoxylin which had been allowed to enter the twigs used, either at the cut end of one of the petioles or through the cut base of the twig. In both instances the evaporation from the leaves furnished a pull which drew the stain into the stem. The haematoxylin was found to follow closely the leaf-trace bundles, showing very little lateral diffusion.

THE PRIMARY VASCULAR SYSTEM OF THE APPLE

Except for the short hypocotyl, the entire stem of the apple tree is the product, primarily or secondarily, of the terminal growing point of the embryo. This apical growing point of the stem is quite
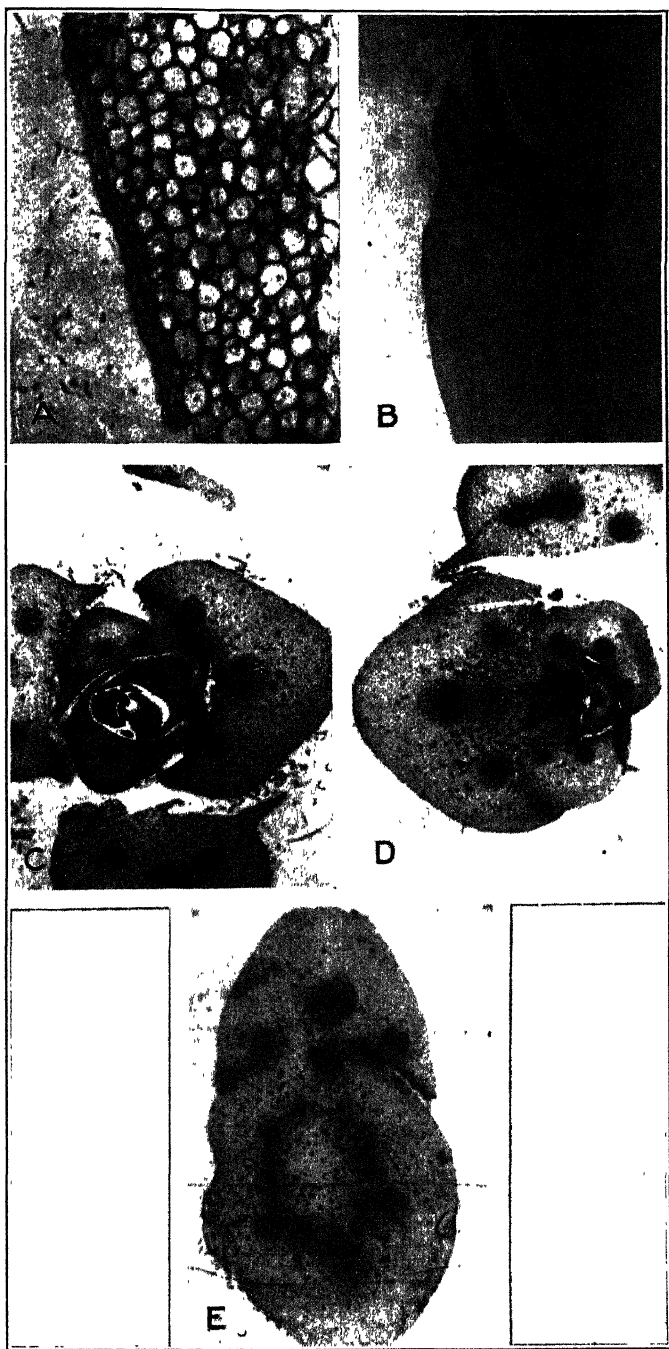
A.—Origin of cork cambium in epidermis. (× about 150)
B.—Longitudinal section through stem growing point   (× 15)
C.—Cross section of stem of Buckskin apple, just above the growing point. (× 15)
D —Another cross section through the stem growing point. (× 15)
E.—Just below the growing point of the stem shown in C and D. (× 15)
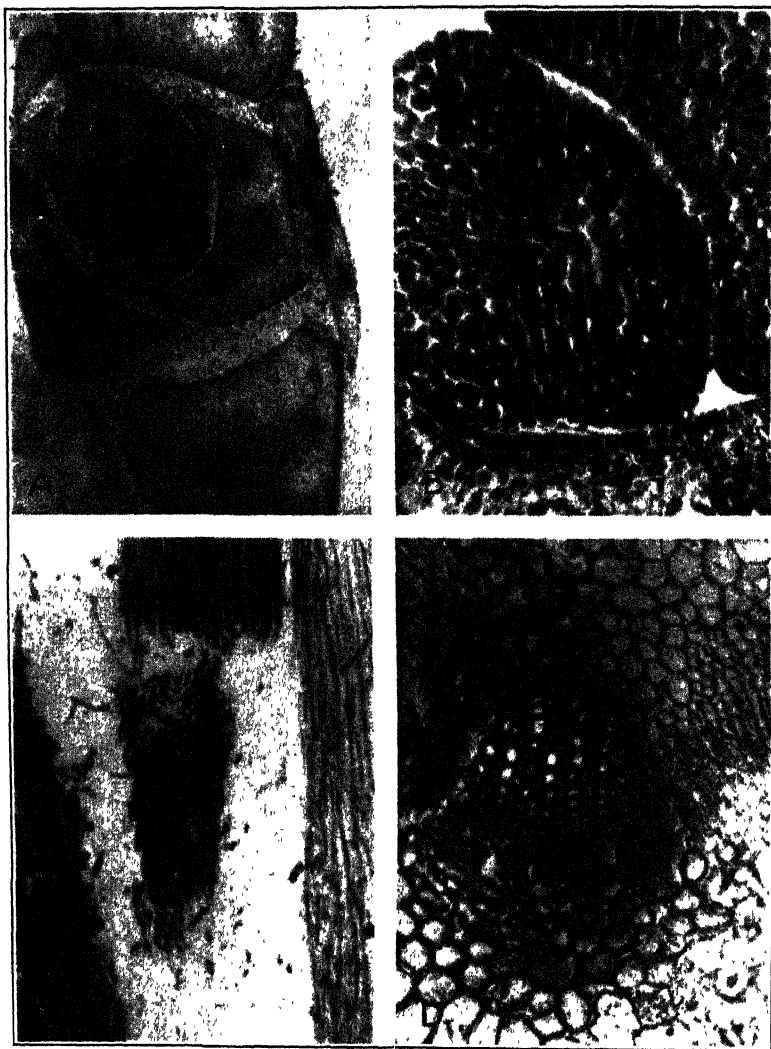
A.—Cross section of the growing point of stem of Wagener apple. Note the procambial strands in the leaves near the center and the vascular bundles, which arose as procambial strands, in the outer leaves. Notice also the protecting hairs. (× about 20)
B.—A higher magnification of part of the stem growing point shown in A. (× 150)
C.—Spiral thickening of primary xylem elements. (× 100)
D.—Origin of cambium within the bundle. Note the metaxylem in radial rows. (× 100)

effectively protected from drying out by the leaves recently formed by the growing point. These leaves grow very rapidly and soon push up and entirely surround the growing point. As will be noticed in Plate 1, B, C, and D, and Plate 2, A, the young leaves bear many hairs.

From this terminal growing point are differentiated the promeristems, from which the tissues of the main stem itself are derived. It also forms the leaves and the lateral branch rudiments or axillary buds. Before primary growth has entirely ceased there has been formed a cambium ring which is to give rise to all secondary vascular tissues. At about this same time the cork cambium, from which the outer portion of the bark is derived, is formed in the epidermis (pl. 1, A). The first cork cambium in most woody dicotyledons is derived from the outer layer of the primary cortex, although not a few genera have a cork cambium initiated in the epidermis, as does the apple.

The terminal growing point of the apple is broad and depressed (pl. 1, B), so that within a few millimeters of the tip the hollow central cylinder or siphonostele, characteristic of most woody dicotyledons, is recognizable. A cross section taken immediately behind the growing point (pl. 1, E) shows pith in the center with the vascular bundles grouped in a ring between a central pith and the cortex. Connecting the central pith and the cortex, and separating the bundles from each other, are the medullary rays. The cells of the central pith, the medullary rays, and the cortex differ from each other chiefly in position. Practically all cells of the stem for several centimeters back of the growing point, even cells of the central pith, contain chlorophyll.

Each bundle of the stem consists of a longitudinal strand of cells. The bundles have no direct connection with the growing point, but as they run upward each passes out of the stele and then on through to the outer cortex, where, coming close to two other similar bundles, it enters the base of the petiole. Here the two outer bundles divide, the inner half of each continuing out into the leaf, the outer half going into a stipule. Followed downward, each stem bundle becomes smaller and smaller in the tangential dimension until—several internodes below the point of its entrance—it merges with a neighboring bundle derived from another leaf. The following description of the leaf traces of woody dicotyledons in general, given by Eames and MacDaniels (6), is applicable to the special case of the apple.

Since the trace is merely an extension of the vascular system of the stem—either as a definite, abruptly separated branch of that system, or as a strand gradually set off as a distinct part—there is not usually a definite point of origin of a trace. The trace, as an identifiable, protoxylem-containing strand may, indeed, often be followed down the stem for some distance below the level at which it begins to swing outward, and there found to merge with other traces or with the xylem of the primary cylinder. . . . Below the point where the trace is separated from the cylinder it is in many cases evident for some distance as a distinct, though not isolated, strand, chiefly of protoxylem. This distinctness of the strand in the xylem cylinder is due to the type and size of cells composing it, these being different from those of the adjacent xylem. . . . Such a downward extension of the trace may be very short or lacking, or may be several internodes in length.

Figure 1 shows diagrammatically the path of the bundles from 9 leaves in a stem of Delicious apple. It will be noted from the diagrams that the general arrangement is such that at any given cross section of the stem the 9 most distinct bundles are the 3 sets
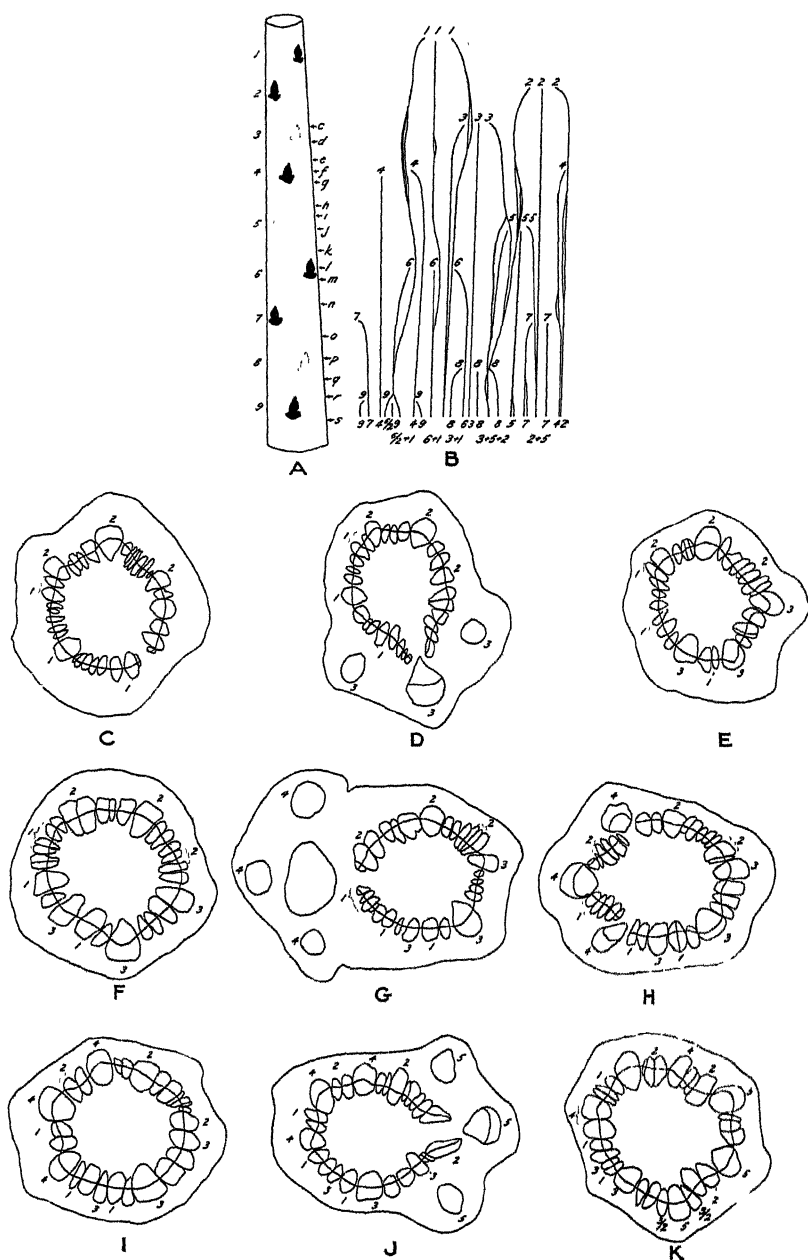
FIG. 1.—Diagrammatic representation of the courses taken by leaf traces in a stem of Delicious apple. A, external appearance of the stem. The numbers refer to the numbers of the buds, the letters to the points at which cross sections having the corresponding letters were cut. B, diagrammatic representation of the paths of the bundles. C to S, camera-lucida sketches of cross sections taken at points indicated by the corresponding letters in A

of 3 each which lead to the 3 leaves next above the section. These bundles are so distributed that one of the side bundles of the uppermost of the 3 leaves (numbered 1 in fig. 1, D) lies between a lateral bundle and the median bundle of the leaf immediately above the section (numbered 3 in fig. 1, D). From the successively lower sections (E to S) it will be seen that the bundles become less and less prominent and that each will ultimately lose its individuality by fusing with a neighboring bundle. This can be clearly seen by following two of the bundles of leaf 2 in diagram B, and in sections C to S. (The third bundle of leaf 2 fused still lower down.)
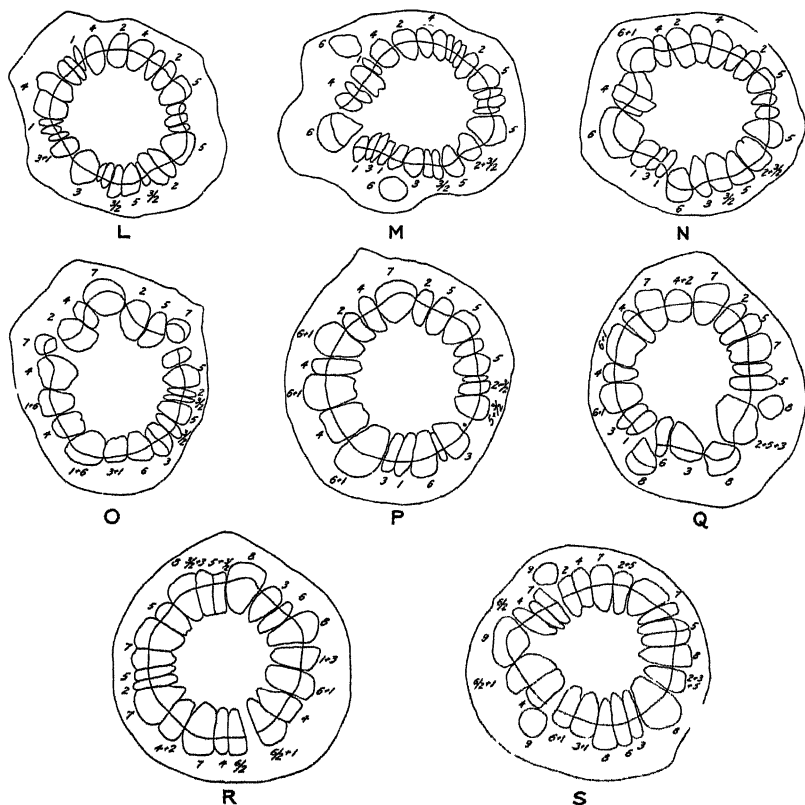
FIG. 1—Continued

Figure 2 shows a series of sections through a Delicious apple stem ɪ which interposition of bundles does not always occur. In rare cases the three traces from each of the three leaves immediately above the section lie next each other in the stele. The laterals of one set do not cross over to become interposed between the laterals and medians of the adjoining set. (See trace 3 at right in diagram B, fig. 2, and sections C to K.)

In other cases, the upper bundle divides, half going on each side of the lateral bundle from the second leaf below. (See trace 2 in fig. 2, B.) In both these sets of diagrams (figs. 1 and 2) it will be
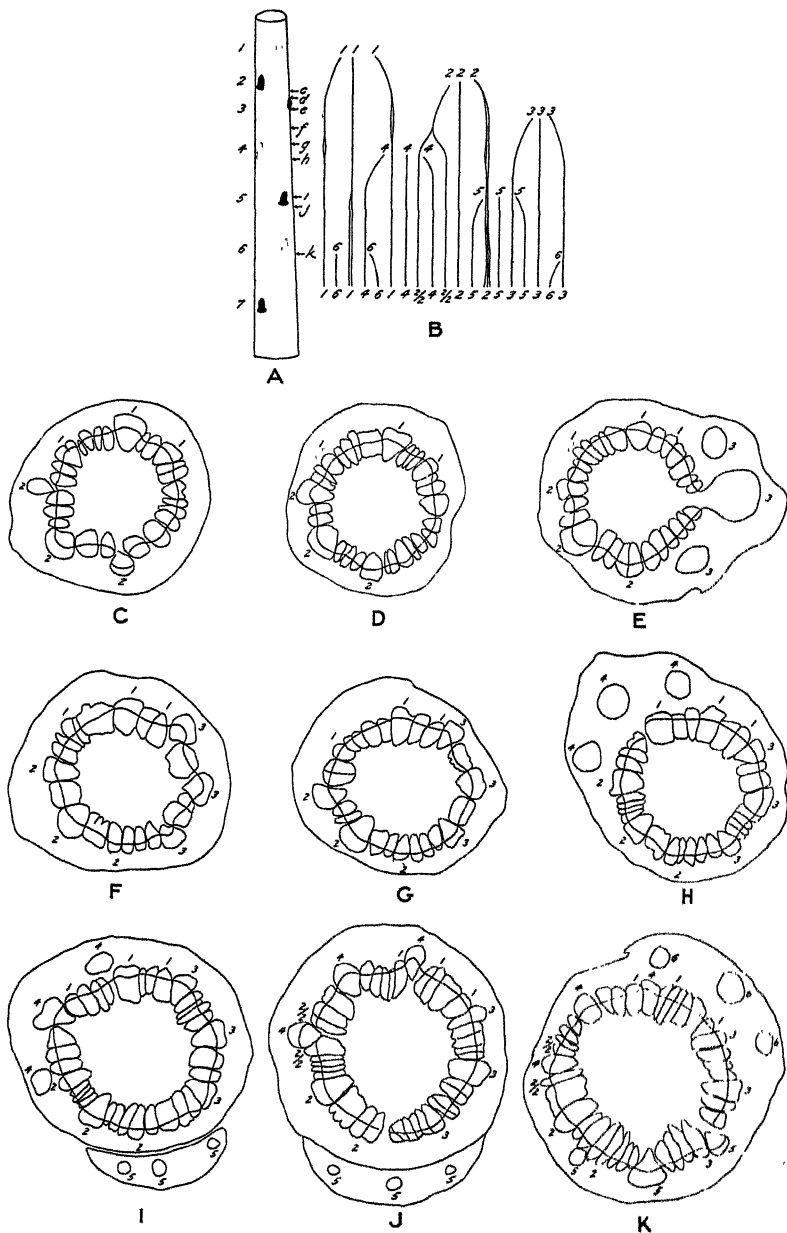
FIG. 2.—Diagrammatic representation of the three types of arrangement of leaf traces found in the stem of a Delicious apple. A, external appearance of the stem. The numbers refer to the numbers of the buds, the letters to the points at which cross sections having the corresponding letters were cut. B, leaf-trace courses. Note that between 3 and 1 there is no interposition such as occurs between 3 and 5. Note also that one of the laterals of leaf 2 divides and goes on both sides of the lateral of leaf 4. C to K, camera-lucida sketches of cross sections taken at points indicated by the corresponding letters in A

noticed that there seems to be little regularity in the way the bundles divide and merge. These irregularities seem surprising in view of the fact that only a primary vascular structure is under consideration and that supposedly it is the genetic constitution, rather than any controllable outside factor, that determines the primary arrangement peculiar to any given species.

As is mentioned below, in connection with the spiral arrangement of leaves on the stem, these irregularities in the courses of the bundles may possibly be significant in regard to the root formation or fruit-bud differentiation or to other factors, though of this nothing is known at present. Nägeli (*10*) noted and diagrammed similar irregularities of the bundle courses in Ribes, Prunus, and in other plants, without commenting on their significance.

The fact that each bundle takes an almost direct longitudinal course and the further fact that merging takes place only after the bundle has become much reduced in tangential width would seem to offer at least a partial anatomical explanation for the physiological results obtained by Auchter (*1*), which led him to conclude that there was normally little or no transverse movement of materials in the apple stem.

After the first differentiation of the leaf rudiment beside the growing point, the bundles of the leaf are laid down as procambial strands. Each procambial strand consists of a group of meristematic cells, each of which divides so that most of the cells are much smaller than those of the undifferentiated part of the growing point. Plate 2, A and B, shows procambial strands formed near the growing point.

By continued division and growth of cells the procambium forms the first water-conducting cells, the protoxylem. These are next to the pith and are closely followed in position and time of development by the metaxylem. Both the protoxylem and the metaxylem persist throughout the life of the plant or until decay sets in at the center of the trunk. These primary xylem elements probably function for many years, although it is only a few weeks before they are in large measure replaced by larger and more efficient tracheids and vessels formed by the cambium. In Plate 2, C, is shown the spiral thickening of the primary xylem with extremely thin walls between the thickened spirals where the thickening bands of some of the elements have been pulled out in sectioning, and resemble very small springs. The larger elements of the metaxylem are pitted and are indistinguishable from secondary tracheids. The apple, unlike most woody dicotyledons, has the primary xylem elements lined up in definite rows, with rows of parenchyma between them. This arrangement makes it difficult, in the stem, to distinguish the metaxylem from the secondary xylem. In the leaf, where no cambium is found, one sees that these same radial rows of metaxylem occur entirely independently of the secondary xylem although they look very much like it.

At about the same time that the protoxylem and metaxylem are being differentiated there arise on the outside of the bundle the protophloem and metaphloem. These differ in appearance from the other cells of the procambium chiefly in being of much smaller diameter. They are living cells, with the function, supposedly, of conducting elaborated food, although this function of the phloem has never been definitely proved, and Dixon (*5*) and others have raised

serious doubts concerning the conduction of elaborated food by the phloem. The primary phloem cells are soon crushed by the outward growth of secondary phloem, cut off by the cambium which arises between the metaxylem and the metaphloem.

In the axil of each leaf either a fruit bud or a branch bud is formed by the growing point. This bud is connected with the pith by a broad band of parenchymatous cells. In contrast to the leaf gaps, the branch gaps extend downward only a short distance. The lateral bud is in all essential respects a duplicate of the terminal bud, but it may or may not have the same leaf spiral that the terminal bud has. If one examines a branched shoot of the apple he will see that all the buds and leaves formed by any one growing point stand in definite relation to one another. Starting at any given leaf and going up the stem through the leaf bases in the order of their origin, one makes two revolutions around the stem, and passes four leaves, before arriving at the sixth leaf, which is directly over the first leaf. Thus the apple is said to have a 2/5 phyllotaxis. It will be noticed, however, that in part of the stems the twist—i. e., the direction of the genetic spiral—is clockwise, while in other stems this spiral joining the leaves in the order of their development runs counterclockwise. It seems improbable that any significant correlation exists between root germ or fruit-bud formation and the direction of the genetic spiral or the twist of the stem. However, it is possible that such a correlation exists, and this point should be investigated.

Between the metaxylem and the metaphloem is a region, at first undifferentiated, but which soon gives rise to the cambium in that portion of the bundle attached to the stele (pl. 2, D). A cambium exists as such for only a very short time, if at all, in that part of the bundle that passes out to the leaf. From the cambium, xylem cells are cut off on the inside and phloem on the outside. Between the vascular bundle strands there is formed the interfascicular cambium, which cuts off parenchymatous medullary ray cells on both sides. Thus, a cross section such as Plate 1, E, shows a complete cambium ring. The cambium layer as a whole forms a hollow, truncate cone, with short breaks at the leaf and branch gaps.

BURRKNOT FORMATION

The majority of burrknots swelling on apple twigs are shown by superficial examination to occur in the immediate vicinity of a bud. Probably this fact led Knight (*8*) to conclude that such burrknots had the power of giving rise to *either* roots or shoots. Since both root and stem growing points are present at the nodes, of course both may arise from almost the same place, although each rudiment has a distinct growing point that probably can develop into only one or the other.

As is shown by Figure 1, C to S, and Figure 2, C to K, the bands (or radial plates) of parenchyma connecting the cortex with the central pith, are, beginning with the broadest: (1) The branch gaps; (2) the leaf-trace gaps; (3) the pair of primary rays, one on each side of each leaf trace after it has entered the stele; (4) the secondary rays within the individual leaf trace bundles. Examination of thousands of sections has led to the conclusion that the order in which they are listed above is also the order of frequency of oc-
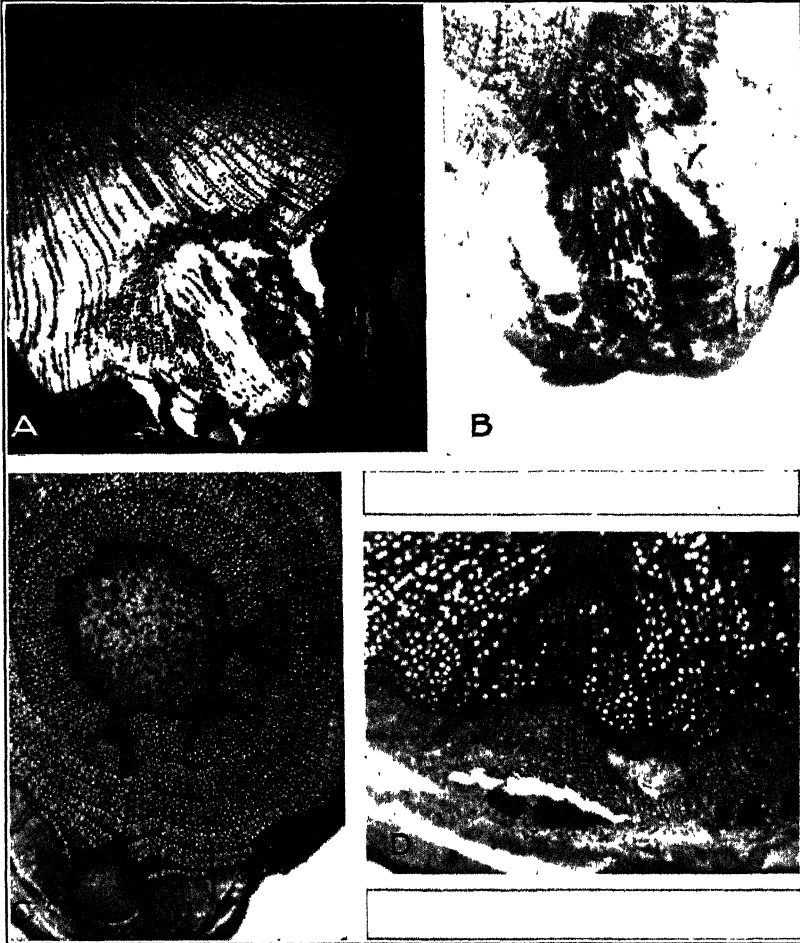
A.—Root germ at node. Note the broad band of parenchyma, characteristic of the branch gap, connecting the root germ with the central pith. (X about 25)
  B.—Root germ, 5 years old, which has arisen on a leaf trace. (See also fig. 3.) (X 25)
  C.—Photomicrograph of cross section shown in diagram in Figure 4, A. (X 10)
  D.—Very young root germ on primary ray of stem of Buckskin apple. (X 25)

currence of root primordia on the four types of rays of apple stems. Figure 3 shows diagrammatically the four ways in which root germs may be placed. Not a single case has been observed on the apple in which roots arose from xylem cells or even phloem cells. Thus the root germs are always placed with definite relation to the primary vascular system, except when they occur on secondary rays.

In the initiation of a root germ, one of the cells in the vertical sheet of parenchyma making up the medullary ray, branch gap, or leaf gap increases its meristematic activity and cuts off a great many cells, such increased activity starting at the point in the ray where it crosses the cambium. No physiological explanation of this strict localization of the root rudiment can yet be suggested. The root germ is soon recognizable in sections as a knob of meristematic tissue. By the end of the second or third year these knobs have elongated and are then roots, with root cap, growing point, cambium, and primary xylem all discernible, as was pointed out by Kissa (7). Apparently the branching of the root is very slight until after the third year. Branching may continue indefinitely, so that on old trees most of the surface of large limbs may be covered with these burrknots. The age at which burrknots appear differs in different varieties. The size of individual root germs, the size of patches of burrknots, and the amount of callus around each root germ also differ.

Fig. 3.—Diagrammatic representation of a radial section of apple stem showing the four ways by which root germs may arise

Plate 3, A, shows a root germ which has arisen on the branch trace. Plate 3, B, shows a leaf-trace root germ several years after differentiation. The root germ has appropriated the vascular system of the leaf trace. This is shown in the diagram given in Figure 3. Figure 4 and Plate 4 show various stages in the differentiation of root germs in the medullary rays bounding the leaf traces and in secondary rays.

WOOLLY APHIS GALLS

Brown (*3*) has called attention to the fact that burrknot swellings might possibly be caused by woolly aphis (*Erisoma lanigera* Hausmann). The writer (*11*) has already published some evidence against this view. His further anatomical work strengthens these objections. A study was made of sections of many twigs which showed superficially the characteristic woolly aphis swelling—a swel-
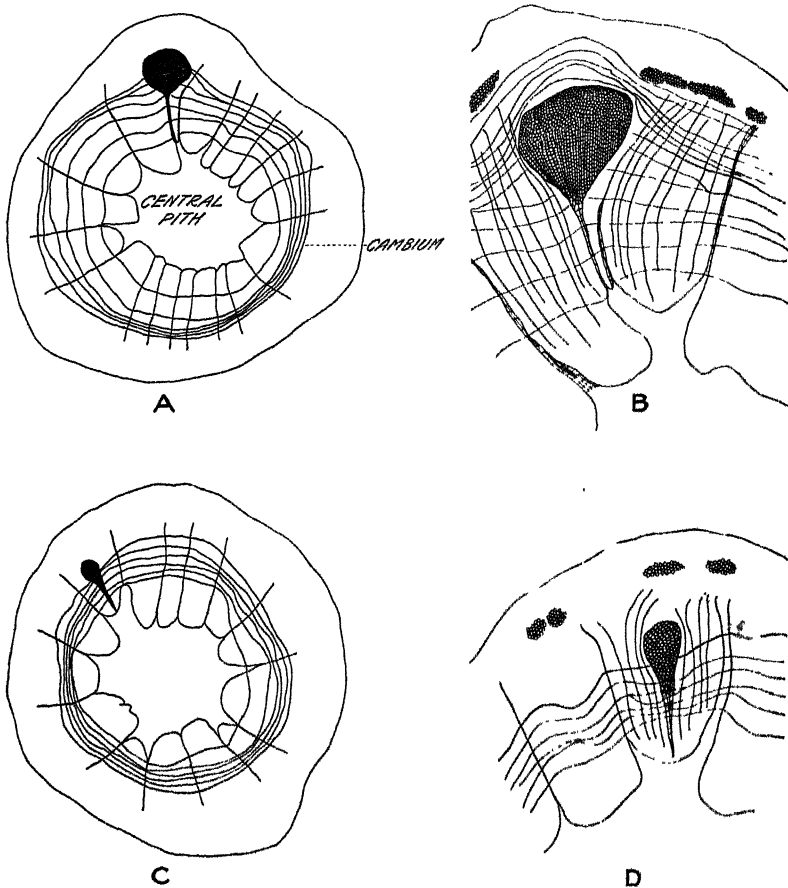
Fig. 4.—A, cross section of 6-year-old Buckskin apple stem, showing root germ which has arisen on a primary medullary ray; B, the same root germ as that shown in A and in Plate 3, C; C, cross section of 6-year-old stem of Buckskin apple, showing root germ which has arisen on a secondary medullary ray; D, the same root germ as that shown in C

ling of a type quite distinct from burrknot swellings. Such (assumed) woolly aphis swellings were taken from the stems of Buckskin, Tolman, and other varieties of apples. Among the varieties at Arlington Farm, the Buckskin showed the most pronounced burrknots, while Tolman has never shown them. The (assumed) woolly aphis galls were about equally numerous on both varieties. As is shown in Plate 5, anatomically the woolly aphis swellings have no points of close resemblance to burrknots. The peculiar anatomical charac-
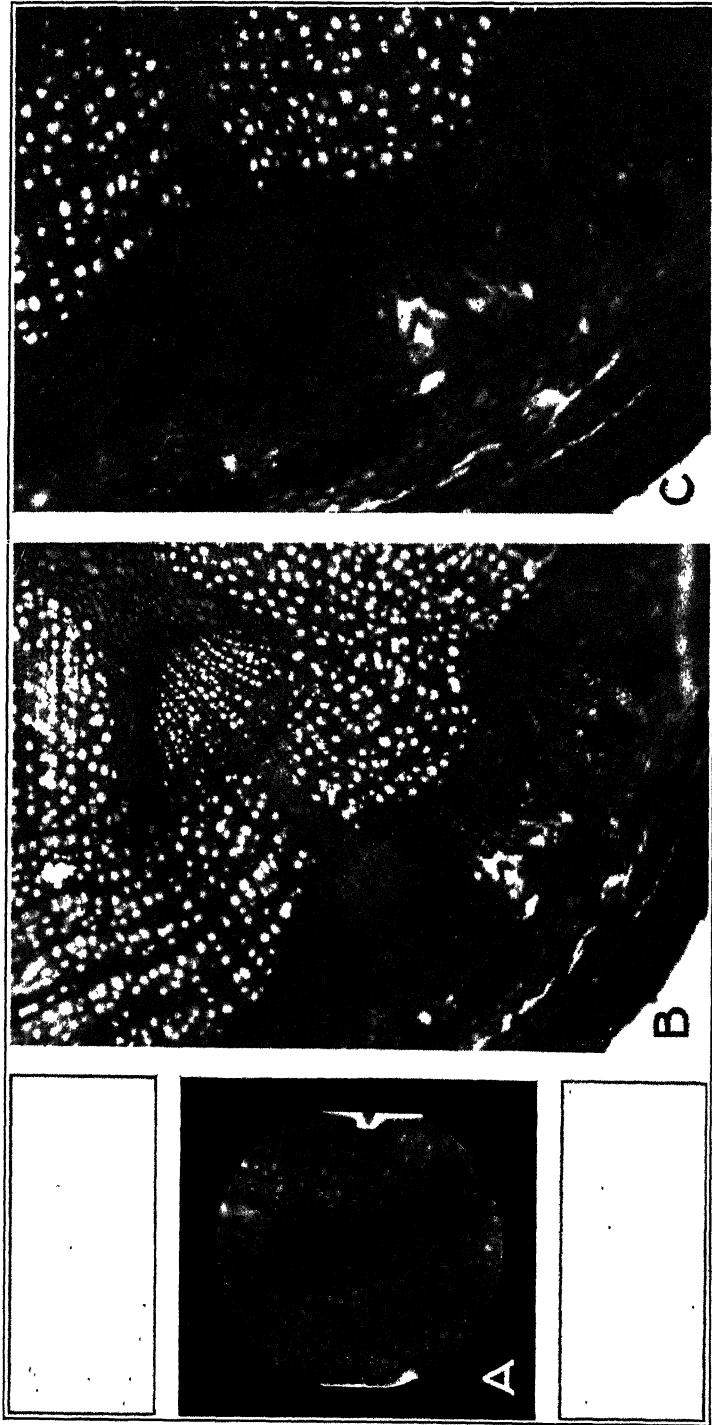
A.—First cell division at base of old root germ. Note that the root arises from one cell of the right row of the primary medullary ray, the left row of cells being pushed aside by the more rapid growth of the root germ. (X about 50)

B.—Root germ arising on a secondary ray. (See fig. 4, C and D.) (X 30)

C.—Same cross section as that shown in B and in Figure 4, C and D. (X 50)

A.—Woolly aphis swelling on 1-year-old stem of Tolman apple. (X about 15)
B.—Woolly aphis swelling on 6-year-old stem of Buckskin apple. Note the very great local swelling of the stem caused by the great increase in the number of fiber tracheids at the close of the second year's growth. Note also the medullary rays that apparently were not changed (except in length) by the woolly aphis. (X 50)
C.—A higher magnification of part of B. (X 200)

teristic of the woolly aphis swellings is a great increase in the number of tracheid fibers produced in the region affected. Approximately the same number of vessels is formed as in the normal stem, but the great increase in the number of tracheids makes it appear that fewer vessels are formed. Nothing was seen which indicated that burrknots ever arise from these woolly aphis swellings.

CONCLUSIONS

From the observations made in the study here reported and those made by other investigators it would seem that the formation of roots on stems has many points of similarity throughout the different plant groups in which it has been studied. Especially in apple and willow the same connection between root germs and the primary vascular system is found. Similar differences exist between different varieties of the same species in regard to the manner and amount of root formation (even apparently its entire absence in some varieties of both apple and willow). The chief differences between willow and apple in regard to the manner and amount of root formation are (1) speaking generally, the root germs on willow arise on younger wood and in greater numbers than those on apple, and (2) the apple root rudiments continue to elongate and to branch more than do those of the willow. With only these minor differences between apple and willow, it seems safe to conclude, in the absence of any contrary proof, either that in both apple and willow the formation of stem-borne roots is due to some pathogenic organism, or that no organism is concerned in either. The writer inclines to the latter view.

SUMMARY

This paper reports a histological study of burrknots in the apple and of the relation of their rudiments to the primary vascular system.

A celloidin-paraffin method of microtechnic is described whereby it was possible to cut, on the rotary microtome, complete series of 8 to 10 micron sections of 10-year-old apple stems.

A method is described of injecting the vascular bundles with dyes, which makes it possible to study in free-hand sections the course of the vascular strands in young apple stems.

The primary vascular system of the apple consists entirely of common bundles, each bundle connected with, i. e., ending above in, a leaf.

Each of the three bundles from every leaf passes down the stem a greater or less distance, ultimately merging with a bundle from another leaf. Before merging, each leaf trace may or may not divide into two or more parts. In some cases the two parts may run down on either side of a bundle entering from a lower leaf.

The general course of the bundles is straight downward in the stem, with few lateral connections. This fact would tend to explain in part the physiological results obtained by Auchter, who found little or no lateral diffusion of material in the apple stem.

The causes for the different courses taken by bundles of successive nodes in the same branch is unknown.

Root germs in the apple may be initiated at the following points in the cambium ring: (1) At the branch gaps, (2) at the leaf gaps, (3) at the primary medullary rays, and (4) at the secondary medullary rays.

The histological structure of woolly aphis galls on the apple is entirely different from that of burrknots.

The origin and arrangement of the root germs of the apple is very similar to the arrangement of root germs in willow, poplar, and other plants. It is concluded that similar physiological and genetic factors (rather than pathological factors) are involved in the formation of root germs in the apple and in the willow.

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FOUR NEW CHALCIDOID PARASITES OF THE PINE TIP MOTH, RHYACIONIA FRUSTRANA (COMSTOCK)[1]

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INTRODUCTION

A large number of parasites of the pine tip moth, *Rhyacionia frustrana* (Comstock), and its variety *bushnelli* Busck, have been reared in recent years by R. A. Cushman and other bureau workers. Among these parasites are four chalcidoids which appear to be new to science, and at the suggestion of Cushman, who is preparing a manuscript dealing with all of the parasites of this host, the following descriptions of these new chalcidoids are published.

FAMILY CHALCIDIDAE

Haltichella rhyacioniae, new species.

Very similar to *americana* (Howard), but may be distinguished from that species by the fact that the first tergite, in addition to being finely striated at extreme base, is finely shagreened over most of the remaining surface. In *americana* the first tergite is striated at extreme base, but otherwise perfectly smooth and polished. The new species differs from *longicornis* (Ashmead) and *onatas* (Walker) in that the scape and all legs are black. *H. xanticles* (Walker) is said to have the first tergite smooth and is considered by Ashmead to be the same as *americana*. *H. perpulchra* (Walsh) is said to have the abdomen glabrous, and the second tergite is apparently short. Types of *americana* and *longicornis* have been examined, but those of the Walker and Walsh species have not been seen.

Female.—Length, 3.60 mm. Head closely punctured all over and clothed with silvery pubescence, the interstices between punctures very finely shagreened; viewed from in front the head triangular; malar space only slightly shorter than the height of eye; antennae inserted at clypeus, nearly three times as long as the height of head, and very slightly clavate; scape long and cylindrical; pedicel about twice as long as thick at apex; third joint of antennae small, quadrate, or nearly so; fourth joint a little longer than second and third combined, about three times as long as thick; fifth to tenth successively decreasing slightly in length, the tenth about one and one-fourth times as long as broad; club ovate, longer than tenth joint but not as long as ninth and tenth combined; dorsum of thorax with pubescence and punctation like head. the punctures not quite as close and the shagreening of interstices a little less distinct; scutellum as long as prescutum, rounded at apex; propodeum gradually narrowing posteriorly, coarsely reticulated and rugulose, with four longitudinal carinae medially, the two nearest the middle strongest and inclosing a subfusiform area, which is three or four times as long as broad and extends from base to apex of propodeum, the two outer carinae curved or angulated and embracing a subcircular or octagonal area within which lies the median area already mentioned; laterad of the large octagonal area on the propodeum are three or four coarse reticulations and the whole surface of the propodeum is finely wrinkled; spiracles large and elongate; venation extending a little beyond middle of wing, marginal vein variable, about twice as long as stigmal; postmarginal very short; hind coxae and femora weakly shagreened and pilose, the femur about twice as long as broad, its ventral margin sinuate, with many very fine teeth; hind tibiae strongly curved, shagreened, its anterior side margined with a delicate carina from base to apex, calcaria two in number, short and unequal; hind basitarsus equal in length to the second tarsal joint; fore and median basitarsus much longer than second joint; abdomen conic-ovate, as long or a little longer than head and thorax; first tergite com-

Journal of Agricultural Research,   Vol. 34, No. 6
Washington, D. C.   Mar. 15, 1927
     Key No. K-187

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prising approximately one-third the dorsal length, almost devoid of hairs, finely striated on its basal one-third, weakly shagreened elsewhere, except a broad smooth border along the posterior margin; second tergite medially about two-thirds as long as first, pilose laterally, shagreened like the first, with the posterior margin and a large rounded area on each side smooth and devoid of hairs; following tergites all more or less shagreened and pilose; third about half as long as second; fourth and fifth short and subequal; sixth about as long as second; seventh as long as third; tip of ovipositor sheath exposed. Black; knees of front and middle legs, apices of all tibiae narrowly, and all tarsi dark reddish testaceous; antennae entirely black; wings hyaline, venation dark brownish.

Male.—Length, 2.80 mm. Antennal flagellum nearly three times as long as scape; scape as in female; pedicel barely longer than broad; flagellar joints thicker than in female; third antennal joint about two and one-half times as long as thick; fourth to tenth successively decreasing slightly in length, the tenth about one and one-half times as long as thick; club no thicker than tenth joint and about one-half longer; abdomen about as long as thorax behind the pronotum, sculptured as in the female, the first tergite usually comprising more than one-third the dorsal length, second about half as long as first, following short and subequal. Otherwise like the female.

Type locality.—Falls Church, Va.
Type.—Cat. No. 40178, U. S. N. M.
Host.—*Rhyacionia frustrana* (Comstock).

Type, allotype, 14 female paratypes, and 15 male paratypes reared in July, 1924, by R. A. Cushman from pupae of the above-named pine tip moth collected on *Pinus virginiana* at Falls Church, Va.; 18 paratypes, including both sexes, were reared in May, 1916, from the same host collected at Falls Church by Carl Heinrich (Hopkins U. S. No. 13924); 5 paratypes were reared from the same moth at Falls Church in 1925 by Cushman; 1 male paratype reared from the same host collected on Nantucket Island, Mass., by I. W. Bailey, in March, 1926. Fourteen paratypes were reared in July, 1924, by Jay Higgins from pupae of *Rhyacionia bushnelli* collected at Halsey, Nebr., and in July, 1925, 20 additional specimens were reared by L. G. Baumhofer from material of the same moth taken at the same place (Hopkins U. S. No. 17508).

FAMILY EULOPHIDAE

Hyssopus rhyacioniae, new species.

This is very close to *H. novus* (Girault), but may be distinguished from that species by its broader face and reddish testaceous hind tibiae. The eyes of *rhyacioniae* are also somewhat smaller than those of *novus* and not so conspicuously hairy, the hairs being shorter. Differs from *thymus* (Girault) in that the pronotum is more slender and the propodeum is without distinct median carina and lateral folds.

Female.—Length, 1.3 mm. Head shining, very faintly shagreened and sparsely clothed with grayish hairs; viewed from in front, very nearly circular in outline; antennae inserted near clypeus; frons a little wider than the vertical length of eye; scrobes moderately deep and subtriangular; eyes oval and thickly set with very short pile; mandibles with small teeth; viewed from above the head is transverse, as broad as thorax at tegulae and about twice as broad as long, the occiput hardly at all concave, temples rounded and immargined, ocelli in an obtuse triangle; postocellar and ocellocular lines subequal; antennae short, clavate; scape slender, not reaching to front ocellus; pedicel longer than thick, as long as first and second funicle joints plus ring joint; first funicle joint small, subtriangular, and about as long as broad at apex; second, third, and fourth funicle joints each about equal to the first in length but successively increasing in thickness, the fourth fully twice as broad as long; club short ovate, thicker than the last funicle joint and about as long as the three preceding funicle joints, practically solid, but with faint indications of a suture beyond the middle; pronotum cone-shaped, a little longer than broad at posterior margin, narrower than the mesonotum at tegulae, shining but weakly shagreened, the sculpture a little more distinct than on the head, sparsely clothed with rather long blackish hairs; mesothorax somewhat flattened dorsally, mesoscutum weakly shagreened,

parapsidal grooves sharply defined; scutellum a little more weakly shagreened than the prescutum and very slightly shorter than prescutum; prescutum with two pairs of black bristles, otherwise bare; scapulae each with one prominent bristle and also sparsely clothed with shorter hairs; scutellum bare except for two pairs of bristles located laterad of the longitudinal grooves; axillae bare; propodeum in nearly the same horizontal plane as the scutellum, practically smooth and shining, without median carina, lateral folds, or spiracular sulci; legs faintly shagreened and hairy; hind tibiae with two distinct but unequal calcaria; forewings reaching beyond the apex of abdomen; marginal and submarginal veins about equal, stigmal approximately one-fourth as long as marginal, postmarginal slightly longer than stigmal; discal ciliation moderately dense except at base of wing, which is mostly bare, but the vestigial median vein is marked by a very distinct row of discal cilia, beginning at base of wing and extending to the posterior margin a little beyond the middle of wing; the area behind this row of cilia is mostly bare except for an incomplete row along the posterior margin of wing and a few much weaker cilia forming an incomplete row between the two; the basal cell is bare; abdomen sessile, about as long as thorax, elliptical in outline, the first segment comprising approximately one-third its length, following segments subequal; venter with numerous bristles toward apex; ovipositor concealed. Black and shining, with a faint bronzy tint on the abdomen; all coxae black, femora black or piceous; all tibiae and tarsi dark reddish testaceous, the apical tarsal joint blackish; antennae piceous, the scape usually dark reddish testaceous; wings hyaline, venation brownish.

Male.—Indistinguishable from the female except by the genitalia and the fact that the abdomen is somewhat shorter and smaller than in the female.

Type locality.—East Falls Church, Va.

Type.—Cat. No. 40179, U.S.N.M.

Type female, allotype, and 17 paratypes reared by R. A. Cushman from larva of *Rhyacionia frustrana* (Comstock) during June and July, 1924.

Elachertus pini, new species.

Nearest to *proteoteratis* (Howard), but may be distinguished at once from that species and also from *coxalis* (Howard) by its black color. Differs from other described North American species by having an abdominal petiole that is fully as long as broad.

Female.—Length, 1.6 mm. Head, viewed anteriorly, distinctly broader than high; eyes prominent, hairy; malar space short; occiput very slightly concave, carinately margined just behind the ocelli but not elsewhere, distinctly shagreened; vertex and front very faintly shagreened; cheeks mostly polished; antennae inserted just above clypeus; scape cylindrical, slightly curved, almost reaching to front ocellus; pedicel longer than broad, a little shorter and not as thick as first funicle joint; first funicle joint slightly longer than broad; the second, third, and fourth funicle joints quadrate; club conic ovate, as long as two preceding funicle joints; pronotum conical but rather short, narrower posteriorly than the mesonotum at tegulae, distinctly shagreened all over and clothed with rather long brownish hairs; mesoscutum short, parapsidal grooves deep, scapulae prominent, prescutum much wider anteriorly than long down the middle; prescutum and scapulae shagreened and clothed with hairs about like pronotum; scutellum nearly twice as long as prescutum, finely and evenly shagreened or reticulated and bare between the grooves, laterad of grooves nearly smooth and with one long seta anteriorly and another posteriorly on each side; axillae weakly shagreened, not produced anteriorly into the posterior margin of mesoscutum but forming with the base of scutellum a nearly straight line; metanotum polished; propodeum with a strong median longitudinal carina, polished and bare medially, very faintly shagreened and clothed with a few long hairs laterad of the folds; marginal vein approximately two and one-half times as long as stigmal; postmarginal about two-thirds as long as marginal; abdominal petiole as long as broad; abdomen beyond petiole ovate, as broad as thorax at tegulae, and somewhat less than twice as long as broad; apex of ovipositor barely showing from above. General color black; vertex, frons, and especially the cheeks and oral region metallic green; thorax faintly tinged with aeneous; abdomen shining black with a pale yellowish spot basally; coxae black; femora mostly piceous; trochanters, tibiae, tarsi, and sometimes the femora at base, yellowish testaceous; wings hyaline.

Male.—Length, 1.4 mm. Funicle joints separated by a distinct short petiole and each clothed with hairs approximately as long as the segment; club terminat-

ing in a very short apical spine; abdomen somewhat shorter than in the female and more rounded at apex. Otherwise like the female.

Type locality.—Falls Church, Va.

Type.—Cat. No. 40180, U.S.N.M.

Five females (one type) and six males (one allotype) reared by R. A. Cushman from *Rhyacionia frustrana* in July, 1924. According to Cushman the species is a gregarious parasite of the larvae.

Secodella subopaca, new species.

In J. C. Crawford's key to species of *Secodella* [2] this runs best to *cushmani* and is very similar to that species, but more deeply sculptured on the mesoscutum and scutellum. In *cushmani* these sclerites are strongly reticulate-punctate, with a strong metallic green and somewhat brassy luster, while in the new species they are uniformly closely punctate and dull bluish green, with scarcely any luster.

Female.—Length, 1.9 mm. Head as broad as thorax, closely punctate and subopaque all over except bottom of antennal scrobe, which is shining; ocellocular line about equal to diameter of ocellus; antennal scape cylindrical, slightly curved; pedicel approximately one-third as long as scape, about one and one-half times as long as thick at apex; flagellum slightly clavate, the funicle joints successively increasing very slightly in width and the club barely wider than the last funicle joint; funicle joints subequal in length, the first slightly longer than pedicel and a trifle shorter than second funicle joint; club about as long as two preceding funicle joints combined, three-jointed, the two basal joints subequal and subquadrate; apical joint very short, conical, and terminating in a short spine; thorax about one-fourth longer than broad; pronotum short, closely punctate; mesoscutum closely and deeply punctate, dull; parapsidal grooves deep and sharply defined; axillae and scutellum sculptured exactly like the mesoscutum; propodeum short, somewhat shining, weakly shagreened laterally but more distinctly sculptured medially, the median longitudinal carina delicate but distinct, lateral folds and spiracular sulci absent; forewings reaching a little beyond apex of abdomen, the discal cilia arranged as usual for the genus but somewhat sparser and weaker than in some of the other species; marginal and submarginal veins nearly equal, postmarginal about one-fifth as long as marginal and one and one-half times the stigmal; hind coxae outwardly rather strongly punctate; abdomen a little longer than head and thorax, distinctly narrower than the thorax, its sides parallel except the apical one-fourth, which is conical; first tergite smooth and constituting about one-fourth the length of abdomen; following tergites all finely shagreened; second, third, and fourth tergites subequal in length, the fifth somewhat longer than the fourth and the sixth still longer than the fifth; apex of ovipositor barely exserted. Head dull bluish in color with a faint tinge of greenish; antennal scape and pedicel concolorous with the head, the flagellum black but covered with dense grayish pubescence; thorax dull blue-green, the pleura purplish in some lights; propodeum and first tergite shining metallic green; tergites beyond the first darker, with a faint metallic sheen; abdomen beneath strongly purplish; wings hyaline, venation brownish; legs concolorous with the thorax except that the basal three joints of middle and hind tarsi are pale and the front tarsi fuscous.

Male.—Length, 1.25 mm. Antennal scape flattened, a little more than twice as long as broad; club not thicker than the funicle, three-jointed, the apical joint terminating in a spine; head and thorax not quite so deeply sculptured as in the female and therefore appearing slightly more shining; abdomen not longer than the thorax, compressed from the sides. Otherwise agrees with the female.

Type locality.—Falls Church, Va.

Type.—Cat. No. 40181, U.S.N.M.

Type female and five female paratypes reared by R. A. Cushman from *Rhyacionia frustrana* (Comstock) collected in the vicinity of Falls Church, Va., in June and July, 1924. The allotype male was reared by Cushman April 17, 1925, from the same host collected at Bogalusa, La., by R. A. St. George.

One female paratype is slide mounted, the others are on card points. The antenna of the allotype is mounted on a slide.

[2] CRAWFORD, J. C. THE GENUS SECODELLA IN NORTH AMERICA. Ent. Soc. Wash. Proc. 17: 142. 1915.

THE CRITICAL TEMPERATURE OF THE CHICKEN [1]

By H. H. MITCHELL and W. T. HAINES, *Illinois Agricultural Experiment Station*

INTRODUCTION

In the course of a somewhat extensive series of investigations of the energy metabolism of chickens and of the net-energy value of feeds for chickens, it became necessary to determine the critical temperature of this animal—that is, the environmental temperature at and below which the heat production of the fasting resting animal will increase to prevent a lowering of body temperature.

REVIEW OF LITERATURE

The first reported investigation of the critical temperature of the chicken appears to be that of Regnault and Reiset, published in 1850 (*10*) [2]. These results, recomputed by Gerhartz (*3*), indicate a continuous decrease in the oxygen consumption of fasting hens as the environmental temperature increases from 19° to 23° C. However, there appears to be no assurance that the activity of the birds was sufficiently controlled in these experiments, a requisite to investigations of energy metabolism the importance of which was not appreciated at this early date.

Gerhartz himself (*3*) has published respiration data from two hens exposed in a fasting condition to external temperatures ranging from 21° to 32° C. The computed oxygen consumption per kilogram body weight per hour, when plotted against temperature, indicated a sharp drop in metabolism at about 23°, followed by a rapid rise at lower temperatures. This temperature was considered to be the critical temperature of the hen. There are, however, several objections to this interpretation. The hens had fasted for only 13 to 24 hours. In the writers' experiments it was found that the effect of food on heat production may extend over a much longer period. Hence, Gerhartz's hens can not be considered as having been in the postabsorptive condition, a condition obviously necessary to a determination of the critical temperature. Furthermore, the sharp rise in oxygen consumption shown by Gerhartz's curve for temperatures lower than 23° may be an effect of individuality rather than of temperature, since the values for 23° and above were obtained from hen 1, while the values below 23° were obtained from hen 2. It is questionable whether values obtained from two animals at distinct temperature ranges can be safely compared in this way.

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[1] Received for publication Jan. 11, 1927; issued May, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 557.

Later investigations on the energy metabolism of geese (*6*), ducks (*7*), and other birds (*4*), aside from indicating more or less certainly a stimulating effect of low temperatures on heat production, have not contributed any definite information as to the critical temperatures of birds.

METHODS AND APPARATUS

In the present study, the respiratory exchange of the hens was determined by means of the gravimetric method of Haldane (*5*). By this method, open circuit in principle, the animal chamber is ventilated with a stream of air that has been rendered dry and free of carbon dioxide by passage through sulphuric acid and caustic alkali (or soda lime). The outgoing air is again passed through these reagents in appropriate containers. The carbon dioxide pro-

FIG. 1.—Apparatus used in critical temperature studies

duction of the animal is measured by the increase in weight of the caustic alkali bottle and of the sulphuric acid container following, while the oxygen consumption is measured by the total increase in weight of the animal chamber and the entire outgoing chain of bottles. An apparatus such as was used by the writers in much of their work with chickens is shown in Figure 1.

Since this particular investigation deals entirely with fasting birds, in which, according to the writers' observation, the respiratory quotient is fairly constant at 0.70 to 0.73, the oxygen determination was dispensed with and variations in heat production were measured by variations in the production of carbon dioxide. The outgoing chain consisted of a Williams bottle next to the chamber, for the removal of water, and a small bottle filled with caustic soda ("shell caustic"), followed by a small gas-washing bottle containing sulphuric acid.

The two last-mentioned containers were much smaller than those illustrated, since the experiments were less than one hour in duration. The smaller size permitted more accurate weighing. Between the Williams bottle and the caustic-soda bottle was a two-way stopcock leading either to the caustic-soda bottle or to the pump directly by way of a rubber tube. By manipulation of this stopcock, the outgoing air could be drawn through the carbon dioxide absorption chain or could be diverted from it.

The experiments were conducted according to the following general plan: All the hens were allowed to fast for at least 41 to 48 hours, since, according to the writers' observation, this length of time is required for the postabsorptive condition to be reached after a normal meal. To avoid any possibility of error, most of the hens were allowed to fast for 72 hours. As soon as a hen was placed in the animal chamber the chamber was sealed with paraffin, ventilation was started, and the stopcock on the outgoing chain was so turned that the air passed directly to the pump. The animal chamber was then brought to the desired temperature by exposing it to a draft of cold air, aided if necessary by the placing of ice on the top of the chamber. The temperature of the air as it emerged from the chamber was taken as the environmental temperature of the hen. The collection of carbon dioxide was not started until the hen had been exposed for at least 30 minutes to the experimental temperature.

In such an investigation as this it is essential to the proper interpretation of the results that the activity of the hen shall be at the lowest possible minimum. This was assured in two ways. The chamber used was just large enough to contain the hen snugly. Any considerable moving around was thus rendered impossible. Furthermore, the hen was in complete darkness, a condition known to favor quietness in hens. For the detection of small movements of the hen, the animal chamber was suspended from a spring. (Fig. 1.) By noting the up and down movement of the chamber, or, better yet, by placing the hand beneath it, slight movements of the hen could be detected. In all of the experiments, the hens were completely at rest, in so far as this condition could be determined by the method used. If, after the air was permitted to pass into the carbon dioxide absorption bottle, any appreciable movement of the hen was noted, the air was immediately diverted to the pump, and was not again admitted to the caustic soda bottle for at least five minutes after the hen had come to complete rest. The time was noted at each turning of the stopcock, in order to obtain the total time of collection, and the rate of ventilation, as measured by a gas meter, was kept constant throughout the period of observation at approximately 3 liters per minute. With these precautions, it is believed that the results obtained possess a definite significance.

EXPERIMENTAL DATA

Twelve Rhode Island Red hens, ranging in weight from about 4 pounds to over 6 pounds, were used in this investigation. Thirty-six observations were made upon these hens, with the results shown in Table 1.

TABLE 1.—*The carbon dioxide production of hens at different external temperatures*

Experiment No.	Hen No	Date of test	Hours of fast	Body weight	Carbon dioxide production per minute at temperatures of— 85° F.	80° F.	75° F.	70° F.	65° F.	60° F.	55° F.	50° F.	45° F.
		1925		*Gm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	2429	Jan. 2	41	2,026				26.8	27.0	23.6	24.0	26.0	
2	2158	Jan. 5	46	2,325					22.5	24.4	30.1	36.0	
3	2420	Jan. 6	46	2,717					26.1	23.1	28.8	30.9	
4	2089	Jan. 7	48	2,500			25.4	26.0	24.8	24.0			
5	2143	Jan. 8	48	2,997			29.1	24.2		24.0			
6	2117	Jan. 9	48	2,233				21.4	21.9	25.6	27.9		
7	2429	Jan. 10	48	1,927					21.9		22.9		
8	2084	Jan. 12	72	2,086			24.5	20.9	23.0				
9	2058	Jan. 14	72	2,509		25.1	30.7						
10	2053	Jan. 15	72	2,710			24.5	27.4	25.9	24.0			
11	2013	Jan. 16	72	2,665			27.2	23.3	28.5		30.0		
12	2000	Jan. 19	72	1,850			19.2	13.8	21.3		22.9		
13	2009	Jan. 20	72	2,485			20.0				24.8		
							19.0				22.4		
14	2158	Jan. 21	72	2,412			25.0				26.5		
							24.8				30.0		
15	2420	Jan. 22	72	2,788			20.0		22.5				
							17.0		25.4				
16	2089	Jan. 23	72	2,625			22.8		20.5				
							21.4		22.0				
17	2143	Jan. 26	72	3,017			24.4		24.2				
							25.9						
18	2117	Jan. 27	72	2,347			17.6		21.1				
							17.5		14.4				
19	2084	Jan. 28	72	2,220			21.7		24.2				
									20.5				
20	2058	Jan. 29	72	2,595			27.8		30.0				
							22.1						
21	2053	Jan. 30	72	2,717			27.2		31.5				
									30.2				
22	2013	Feb. 2	72	2,749					20.5		35.1		
									24.4		27.3		
23	2420	Feb. 3	72	2,809							22.0		24.0
											21.6		27.0
											22.0		
24	2000	Feb. 4	72	1,905							24.8		26.9
											20.5		27.5
25	2009	Feb. 5	72	2,562	20.7		24.7						
					20.0		22.7						
26	2158	Feb. 6	72	2,657	24.3		21.1						
					21.0		21.2						
							22.5						
27	2429	Feb. 9	72	2,145	27.5		18.7						
					23.0		18.4						
							19.5						
28	2089	Feb. 10	72	2,685				24.4		20.2			
								19.2		28.8			
29	2143	Feb. 11	72	3,042						30.6	30.7		
										28.0	35.8		
30	2117	Feb. 12	72	2,430						17.4		24.8	
										19.2		24.0	
31	2084	Feb. 13	72	2,387						29.8		26.1	
										26.4		29.5	
32	2058	Feb. 16	72	2,745						24.3	29.7	37.0	
										27.7			
33	2053	Feb. 17	72	2,875						30.0		30.4	
										28.5		35.3	
34	2013	Feb. 18	72	2,970						26.6		26.0	
										25.9		31.4	
35	2420	Feb. 19	72	2,940						24.7		26.4	
										22.7		27.2	
36	2000	Feb. 20	72	2,062					21.8	24.7		28.1	

Environmental temperatures ranging from 45° to 85° F. were imposed, and in many of the experiments duplicate or triplicate determinations of the carbon dioxide production were made at each of the temperatures used. The order in which the different temperatures were imposed is not indicated in the table, since, from a study of the records, it did not appear to be a matter of consequence.

Evidently the individual experiments were not planned—at least in most cases—in such a way that the exact location of the critical

temperature could be determined. The most that any one experiment indicates is that the desired temperature is above or below a given point. Therefore in their most complete interpretation, they must be considered in the aggregate. The experiments have been summarized in Table 2, according to the following scheme:

TABLE 2.—*The number of experiments which indicate the location of the critical temperature either above or below the environmental temperatures imposed*

Temperature imposed	Number of experiments locating the critical temperature—	
	Below the temperature imposed	Above the temperature imposed
°F.		
45	0	23
50	1	21
55	3	17
60	6	12
65	12	8
70	13	2
75	14	2

1. If the results in experiments involving only two temperatures indicate, more or less certainly, that the production of carbon dioxide per minute was greater at the lower than at the higher temperature, they are interpreted to mean that the critical temperature is above the lowest of the two temperatures. Its location with reference to the highest temperature can not be inferred. In experiment 13, for example, the two observations at 75° F., i. e., 19 and 20 mgm., are lower than those at 55°, i. e., 24.8 and 22.4 mgm. Hence, the critical temperature is evidently above 55°, but whether it is above or below 75° the results do not indicate. Since, in this experiment, the critical temperature is above 55°, it must also be above 50° and 45° and is so listed in Table 2, where it is entered at these three temperatures.

2. Similarly, if a series of continuous observations extending over more than two temperatures indicate a continuous increase in carbon dioxide production with each decrease in temperature, the conclusion is justified that the critical temperature is above the second highest temperature of the series. Experiment 2 is a good example of this type. A continuous increase in carbon dioxide production was observed from 70° to 55° F. It is evident that the critical temperature in this experiment is above 65°, but whether it is above or below 70° can not be decided, since the increase in carbon dioxide production observed may have started either at a point between 65° and 70° or at some point above 70°. In this case, therefore, it can only be concluded that the critical temperature is above 65°, and hence above all lower temperatures. In Table 2, therefore, this experiment is listed as indicating a critical temperature above 65°, 60°, 55°, 50°, and 45°, thus entering the table in five places.

3. If a series of two or more continuous observations at different temperatures shows no significant effect of environmental temperature on carbon dioxide production, the conclusion is justified that the critical temperature is below the lowest temperature of those at which observations were taken. An illustration of such an experiment is afforded by experiment 10, which indicates a critical temperature below 60° F., experiment 16, which indicates a critical

temperature below 65°, and experiment 31, which indicates a critical temperature below 50°. Experiment 10 was used in the construction of Table 2 as indicating a critical temperature below 75°, 70°, 65°, and 60°; experiment 16, as indicating a critical temperature below 65°, and all higher temperatures; and experiment 31, as indicating a critical temperature below 50° and all higher temperatures.

4. If, in a series of observations extending over several temperatures, it appears that the carbon dioxide production of the hen increases somewhere between two of the temperatures chosen, it is inferred that the critical temperature is similarly located. Experiment 6 represents such a case, and in the construction of Table 2 is taken as indicating a critical temperature above 60°, 55°, 50°, and 45°, and below 75°, 70°, and 65°, thus entering the table at seven places. Experiment 11 is similar.

5. In a few of the experiments, a tendency to an increase in carbon dioxide production at the higher temperatures is evident. Since such an increase bears no relation to the critical temperature, it is not considered in the construction of Table 2. Such an increase may be noted in experiments 5, 11, and 27.

Having considered the method used in summarizing and evaluating the results in Table 1, the results given in Table 2 may now be studied with the idea of obtaining an average value for the critical temperature of the chicken. None of the experiments indicated a critical temperature below 45° F., while 23 indicated one above this temperature. For 50°, one experiment pointed to a critical temperature below, while 21 pointed to a critical temperature above. The figures in the two columns at the right approach equality between 60° and 65°. For observations taken above 65°, the evidence points unmistakably to the conclusion that the critical temperature is below this range rather than above. The evidence as a whole, therefore, may be taken to point to an average critical temperature for the Rhode Island Red hen of approximately 62° F. or 16.5° C. Since these birds were taken from the university poultry farm in the middle of winter, this critical temperature may be considered as applying to chickens under winter conditions of feathering and under the condition of low humidity obtaining in the animal chamber.

This average critical temperature of 16.5° C. for the winter-feathered Rhode Island Red hen may be compared with the critical temperature of about 21° C. for the pig, as recently determined by Capstick and Wood (*1*). Forbes and his associates (*2*) have reported a critical temperature greater than 18.3° C. for a steer with closely shorn hair and less than 15.5° for a steer carrying a full coat of hair. Morgulis (*9*) determined the critical temperature of a female Irish terrier before and after clipping to be, respectively, between 13.6° and 15.1°, and between 23.8° and 26.5° C. The critical temperature of the goat, determined by Magee, (*8*) is not comparable to the values above given, since the animal was not fasting but was receiving approximately a maintenance ration. Under this condition, the critical temperature appeared to lie between 12.7° and 21.1° C. In the fasting state the temperature would be higher still.

A study of the individual reactions of the 12 hens used in the metabolism experiments herein reported reveals considerable differences. The best judgment of the writers as to the location of the critical temperatures of the individual birds is given in Table 3.

TABLE 3.—*Probable location of the critical temperatures of the individual hens*

Hen No.	Number of experiments	Probable location of critical temperature (° F.)
2429	2	Below 55.
2158	3	Between 65 and 75.
2420	4	Above 65.
2089	3	Inconclusive.
2058	3	Above 75.
2013	3	Between 65 and 75.
2000	3	Above 65.
2009	2	Above 75.
2084	3	Below 50.
2143	3	Between 55 and 60.
2117	3	Between 60 and 65.
2053	3	Inconclusive.

The reaction of hen 2084 stands in marked contrast to that of hens 2058 and 2009. Such differences may be due to differences in feather covering or in the amount of subcutaneous fat, or in the vasomotor control of the birds. They represent the penalty of working with several animals rather than with one, in so far as the consistency of the data obtained is concerned.

Although the practical importance of the location of the critical temperature of the fasting farm animal is obvious, it should be realized that it is subject to variations, not only in the animal itself, but in its thermal environment. At constant low temperatures the heat production of an animal may vary with the humidity of the surrounding atmosphere or with the prevalence and intensity of air currents. It is the cooling power of the surrounding air, rather than its temperature alone, that determines whether the heat produced in metabolism is sufficient to maintain body temperature. Investigations concerned with the relative importance of temperature, humidity, and air movement would be of great interest and value.

RATE OF INCREASE IN HEAT PRODUCTION OF ANIMALS EXPOSED TO ENVIRONMENTAL TEMPERATURES BELOW THE CRITICAL

Another problem of importance in this connection relates to the rate at which the heat production of an animal will increase per degree fall in the environmental temperature below the critical. It is known that above the critical temperature the emission of heat from the body surface is regulated by vasomotor reflexes, by reflex stimulation of the sweat glands, and at high temperatures by the type of breathing. If it may be assumed that at the critical temperature the radiating capacity of the skin is reduced to the lowest possible minimum by vasoconstriction of the arterioles feeding the subcutaneous capillary bed, and that the secretory activity of the sweat glands has been totally inhibited, it is reasonable to suppose that Newton's law of cooling bodies will apply, and that

$$H = k \ (t - t^1),$$

where H is the heat emission (or heat production) at the critical temperature, t is the normal body temperature of the animal, t^1 the critical environmental temperature, and k a constant equal to the increase in heat emission (or heat production) per degree drop in temperature. If this equation represents the conditions prevailing,

the increase in heat production with decrease in temperature below the critical should be linear.

The only experimental data that appear to be available for testing the applicability of this equation to animals in environments below the critical are those of Capstick and Wood on the pig.[3] These data indicate a linear increase in heat production with decreasing environmental temperatures below the critical, as the equation would predict. The experimental data also indicate that the increase occurred, in the particular pig observed, at the rate of 0.077 calorie per minute per degree drop (*1*, *p. 265*). It is interesting to compute the rate of increase predictable from the above equation. At 20.4° C. (the approximate critical temperature), the basal heat production of the pig, corrected to a weight of 300 pounds, was 1.499 calories per minute. If the body temperature of the pig be taken as 39.5° C. (*11*, *p. 90*), the equation becomes

$$1.499 = k \ (39.5 - 20.4)$$
$$k = 0.078 \ \text{calorie.}$$

This value of k, computed from a theoretical consideration of the conditions prevailing at the critical temperature, is in remarkably close agreement with the value experimentally determined (0.077 calorie) from observations at and below the critical temperature. This agreement, together with the linear character of the rise observed in the heat production as the temperature fell below the critical, may be taken as legitimate confirmation of the applicability of Newton's law of cooling bodies to the relation between the basal heat production of the pig and the environmental temperatures at and below the critical.

If the formula may be assumed to apply to the hen also the practical significance of the value of k may be illustrated as follows:

A 5-pound hen, according to unpublished data, will possess an average basal heat production of approximately 115 calories per day. If the body temperature of the hen be taken as 106° F. and the critical temperature as 62° Newton's formula becomes

$$115 = k \ (106 - 62), \ \text{and}$$
$$k = 2.6 \ \text{calories per degree Fahrenheit.}$$

This means that for each drop of 1° F. in the temperature of the environment below the critical, the heat production of the hen must increase 2.6 calories per day in order to maintain her body temperature. If the hen is moving around actively in a small pen, the writers' data indicate that her heat production may easily be increased by 70 calories daily; the critical temperature of the fasting *active* hen may be computed to be $62 - (70 \div 2.6) = 35°$. If the hen is consuming a ration composed largely of corn, sufficient in amount to support an egg production of one per day (estimated to be 0.217 pound), the heating effect of this amount of food may be estimated, from unpublished data, at 51 calories. This amount of heat is sufficient to cause a further drop in critical temperature of about 20°. Therefore, it may be roughly estimated by the use of Newton's con-

[3] In no one of the experiments reported in Table 1 was the critical temperature of the hen located with sufficient definiteness to permit its use in this sort of a calculation.

stant that a 5-pound hen confined to a small pen and receiving a sufficient ration, based mainly on corn, to support the production of one egg a day, could stand an outdoor temperature of 15° before requiring additional food to keep her body warm. Evidently this rough prediction would be too low if the atmosphere were excessively humid, increasing the heat loss from the body by conduction, or if the hen were exposed to a brisk wind, which would have the same effect.

SUMMARY

From 36 experiments with 12 Rhode Island Red hens, involving 137 determinations of the carbon dioxide production during fast and quiescence at different temperatures, it was found that the average critical temperature was 62° F. This value applies to winter-feathered birds in an atmosphere of low humidity and in an air current of approximately 3 liters per minute. Some of the individual birds appeared to exhibit distinct differences in their reaction to changes in environmental temperature.

It is shown that Newton's law of cooling bodies may apply to animals at environmental temperatures below the critical. The practical significance of this conclusion is discussed and illustrated.

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HEREDITARY AND ENVIRONMENTAL FACTORS THAT PRODUCE MOTTLING IN SOY BEANS [1]

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INTRODUCTION

Mottling in soy beans has been considered as an inherited character and one that indicates genetic impurity. For this reason all soy-bean seeds blotched with irregular patches of black or brown pigment have been regarded as objectionable by most farmers and seed dealers. For the same reason the International Crop Improvement Association has recommended that all mottled soy beans be looked upon as "mixed," that they be ruled out of "registered seed," and that they be tolerated to the extent of only 1 per cent in "certified seed."

So pronounced has been the unpopularity of mottled soy beans that many efforts have been made to eliminate mottling by breeding and selection. In some instances these efforts seem to have been successful, but in certain sections of the country mottling has persisted in spite of all attempts to overcome it. This fact has been surprising to those familiar with the uniformity of colorless or non-mottled varieties of the common bean (*Phaseolus vulgaris*), and the cause of the peculiar phenomenon of mottling in soy beans has puzzled technical investigators as well as practical growers.

The purpose of the present study was to determine how far one is justified in discriminating against mottled soy beans and to ascertain if possible the factors which cause the mottling. There is much yet to be learned concerning the fundamental physiological processes involved in mottling, but the results so far obtained show that environment as well as heredity plays a very important rôle in the process. This knowledge may be of some value to those who are concerned with the practical bearing of the question, and it is also hoped that the observations herein reported will stimulate others to further research.

OCCURRENCE OF MOTTLING AND THE WORK OF PREVIOUS INVESTIGATORS

In the United States the problem of mottling has been discussed in meetings of the National Soy Bean Growers' Association, and work from a number of stations has been presented. At the meeting of this association in September, 1925, J. B. Park, of the Ohio Experi-

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 6
Mar. 15, 1927
Key No. Wis-40

(559)

ment Station, gave a summary of the work reported on mottling up to that time. He referred chiefly to the graduate work of J. S. Culter at Ohio State University and of E. A. Hollowell at Iowa State College. Park emphasized the fact that whereas mottling has been found to be of very common occurrence in some parts of the country, in other sections it is almost unknown.

Hollowell's thesis [3] covers the results of three years' work. From tests which he supervised in various parts of Iowa he concludes that mottling may be greatly affected by soil conditions but not by climatic conditions. He found that more mottling occurred on rich soils than on soils deficient in fertility and that plants grown close together produced less mottling than plants spaced some distance apart in the row. Hollowell's work has much in common with that carried on by the present writer at Wisconsin.

In the Orient, where the soy bean has long been an important crop, no particular study of mottling has ever been made so far as the writer is aware, but from all reports mottling is also of common occurrence there. Nagai (*8*, *9*)[4] has done considerable work in Japan on the inheritance and chemistry of soy-bean pigments, but his studies will be referred to in a later part of this paper.[5]

PIGMENTS WHICH PRODUCE MOTTLING

Two pigments are primarily concerned with the production of seed-coat colors in soy beans, namely, black and brown. In black seed coats there is frequently considerable brown present in the spongy parenchyma cells (pl. 1) and in certain cases there is undoubtedly a mixture of the two pigments in the Malpighian cells. In mottled seed coats the pigments are the same as in those of self-colored varieties, the only difference being the complete development of the pigment to produce the self colors and the restriction of pigment in the mottled types. Data which are not entirely conclusive show that the seed coats of black or brown varieties are heavier than those which are free from these pigments, indicating that the pigments have an appreciable weight. No significant difference, however, has been found between the weight of mottled and nonmottled seed coats.

The black pigment is bright red for some time before the seed matures. Then as the seed becomes desiccated the color changes to purple and gradually becomes intensified. The brown pigment first appears as very light pink, but this is well toward the period of maturity, and the typical brown color is not apparent until the seed is ripe.

The black, or deep purple, pigment turns red in acid solution, and has all the general properties of anthocyanins. It is precipitated by salts of heavy metals and turns brown or green in alkaline solution, the exact shade depending on the alkali used. Hydrogen peroxide or sodium sulphite decolorizes a solution of the pigment very rapidly. It is very slightly soluble in pure water and almost entirely insoluble in 95 per cent alcohol, but either of these solutions becomes a fairly good solvent when made slightly acid. A 50 per cent solution of

alcohol containing 1 per cent hydrochloric acid is an excellent solvent. Acetone is also a good solvent, especially when slightly acidified, but propyl and butyl alcohols are rather poor solvents for extracting the pigments from the tissue of the seed coat.

Except for a difference in color, most of the chemical reactions of the brown pigment are very similar to those of the black. Nagai (8) has used the term "phlobaphene" in connection with this brown pigment, as well as with the brown pigments which occur in many other plants. He found that this term dates back to Stähelin and Hofstetter in 1844, but it has been a general term applied to brown pigments known to be derived from tannins. The exact chemical . nature of most of these substances is not known, but since Sando and Bartlett (16) have identified an isomer of quercetin in the brown husks of Emerson's maize it would not be surprising to find that many other brown pigments are also flavone glucosides.

Neither the black nor the brown pigment is soluble in ether, chloroform, carbon disulphide, or similar solvents; and ether precipitates them when added in excess to an alcoholic solution. This should provide a means of purification, but so far the writer has not been successful in getting either pigment in a pure crystalline form. One difficulty is perhaps due to the fact that there is a mixture of the two pigments in black seed coats. It is also possible that various types of flavone and flavonol glucosides may be present as mixtures.

It is doubtful whether there are but two pigments primarily concerned in seed-coat colors of soy beans, yet the situation seems much simpler than with many other legumes. In species of Phaseolus the color types are much more complicated, and while anthocyanin-like pigments probably cause most of the coloration, there must be numerous modifications to produce the various shades of reds, browns, and purples that are found. In the cowpea, Mann (6) found that black and brown melanin pigments occur as well as anthocyanin; and, in general, the seed coats of legumes are well known for their diversity of color types and patterns.

Besides the regular black and brown pigments in the seed coats of soy beans, there is an oxidation pigment which is frequently associated with the seed coats of black-podded varieties. Sometimes it produces a smudgy appearance, especially when the pod and testa are injured, but it is interesting to note that this pigment is localized chiefly in the parenchymatouslike cells of the seed coat, while the common black and brown pigments are largely restricted to the outer Malpighian cells. Since this pigment is not related to the brown and black pigments, however, no further mention will be made of its occurrence.

Another type of pigment has also been observed by Matsumoto and Tomoyasu (7) which is caused by a fungus (*Cercosporina kikuchii*) that is parasitic in the seed coat of the soy bean. Considerable purple mottling and seed injury are reported in certain instances as a result of the action of this organism.

FACTORS KNOWN TO INFLUENCE THE DEVELOPMENT OF ANTHOCYANIN PIGMENTS

Onslow (11) concludes that anthocyanin pigments are very widely distributed in the plant kingdom, and the species that do not become colored to some extent under suitable conditions are rare. Certain

environmental conditions are known to favor the production of pigment, and if these are not provided the pigment-producing potentialities of a plant may not become apparent. Since the anthocyanin pigment in the seed coat of the soy bean is very similar in chemical behavior to anthocyanins which occur in other plants, considerable may be learned from a consideration of the factors which are known to favor the production of these pigments.

Among the factors other than heredity that are responsible for the development of anthocyanin, light, temperature, oxygen supply, drought, and altitude have all been elaborated upon, but the most important fundamental factor seems to be the accumulation of synthetic products, especially sugars.

MacGillivray,[6] working with tomato plants, found that pigmentation was intimately associated with the phosphorous supply. When phosphorus was not available there was a very noticeable decrease in the production of nitrogenous compounds and this caused his plants to become highly colored. Under such conditions it seems natural that plants should be high in carbohydrates, and analyses showed this to be the case.

Since anthocyanins are glucosides, one would expect carbohydrates to be very essential for their formation; and Onslow's (*10*, *11*) work shows that the form in which the carbohydrates occur is also important. Overton (*12*), Katić (*4*), and Klebs (*5*) previously found that certain species of plants develop pigment very quickly when fed on sugar solutions. Cuttings of land plants were used in their experiments as well as water plants. Onslow (*10*), continuing this study, found that after she had fed a 3 per cent cane-sugar solution for 7 to 10 days, certain species of land plants developed pigment and others did not. A test for starch was then made on all the species under observation. Again it was found that some species formed starch and others did not. The interesting point in this connection is the high negative correlation that was found between starch and pigment formation. In species that were not able to synthesize starch, sugars accumulated; and since sugars are used in the formation of anthocyanins, a logical reason can be given for pigmentation. Plants that were able to form starch, she assumes, made use of their sugars in that process, and the quantity of sugar left was insufficient for the production of pigments.

A great deal of space would be required to review the observations published on the relation between the accumulation of sugars and pigment formation. Suffice it to say that the physiological condition of the plant is apparently the ultimate determining factor. In many cases the problem becomes very complicated. Especially is it difficult to distinguish factors that are directly concerned with pigment formation from those that are only indirectly concerned in that they affect the food supply of the plant.

Two of the outstanding factors which have been studied are light and temperature. Light is very probably a critical factor in the direct chemical processes that bring about certain anthocyanin pigments. Temperature also seems to be an important factor in certain instances, but it is difficult to distinguish its direct influence on pigment formation from the indirect influence which it exerts through

[6] MacGillivray, J. H. EFFECT OF PHOSPHORUS ON THE NUTRITION OF THE TOMATO PLANT. 1925. Unpublished doctor's thesis. Copy on file, library, Univ. Wisconsin, Madison.]

its effect on the accumulation of food material in the plant. For a more detailed treatise on this subject the reader is referred to Onslow's (*11*) "Anthocyanin Pigments of Plants," which also contains an excellent bibliography.

ENVIRONMENTAL FACTORS IN RELATION TO MOTTLING

To find the determining factors in the production of mottling, limited studies have been made of plants grown under artificial conditions, but the most striking results have been obtained by carefully observing the behavior of plants in the field. These observations have confirmed the conclusion of Hollowell [7] that rich soils and a liberal distance between plants are usually conducive to mottling. Different soils have produced striking differences in the production of mottling, but the variability on the same type of soil and even on the same plant has also thrown much light on the cause of mottling. These studies have given conclusive evidence that environment affects the production of mottling and also that a delicate nutritional balance exists which likewise influences it.

Growing plants under different conditions and studying the variations which occur have contributed much information to the general problem, but the most valuable findings have been obtained from certain abnormalities.

DISTRIBUTION OF MOTTLED SEED ON THE PLANT

Woodworth and Cole (*18*) found that the distribution of mottled soy-bean seed may be extremely variable. They observed striking resemblances among seeds borne on the same plant, but breeding results did not indicate that these were due to heredity. They concluded that physiological factors must have been responsible for the results obtained.

In this discussion it may be well to remind the reader that the pigments under consideration are formed in maternal tissue. The portion of the seed coat in which the pigment occurs is derived from the outer integument of the ovule and is independent of the embryo, which belongs to a new generation. Hybridization, of course, may affect the embryo immediately, but the seed coat begins to form before fertilization, and for this reason all the seed coats of any one plant should be genetically identical, no matter how much hybridization has taken place. For this reason hybrid seeds can not be detected by any difference in the seed coat until the seeds are grown and another generation of seed coats is produced. If a pollen grain from a black variety of soy bean chances to fertilize an ovule from a brown variety the hybrid seed will still be brown and can not be distinguished on the basis of seed-coat color from any of the other seeds borne by the same plant; but when the hybrid seeds are grown and produce F_1 plants all the seeds borne by one of these plants will be black instead of brown. In this way the seed coat is always one generation behind the cotyledons because the cotyledons are part of the new embryo and may be immediately affected by hybridization.

Since all the seed coats on one plant are made up of cells which supposedly have the same hereditary potentialities, one would

[7] HOLLOWELL, E. A. FACTORS INFLUENCING THE MOTTLING OF THE SOYBEAN SEED COAT. 1924. [Unpublished master's thesis. Copy on file, library, Iowa State College, Ames.

expect all to be the same color or pattern if these depended entirely on the hereditary constitution. If the variation in mottling were found to be inherited, it would be necessary to assume that somatic mutation or some other change has taken place in the genetic constitution during sporophytic development. This has been found to explain the variation of the mosaic pericarp of corn (*2*); and Nagai (*9*) has obtained results which indicate that soy beans may not be entirely free from somatic mutations. However, the writer now has a great many data which show that heritable somatic changes are not the cause of the immense amount of variation in the production of mottling in soy beans.
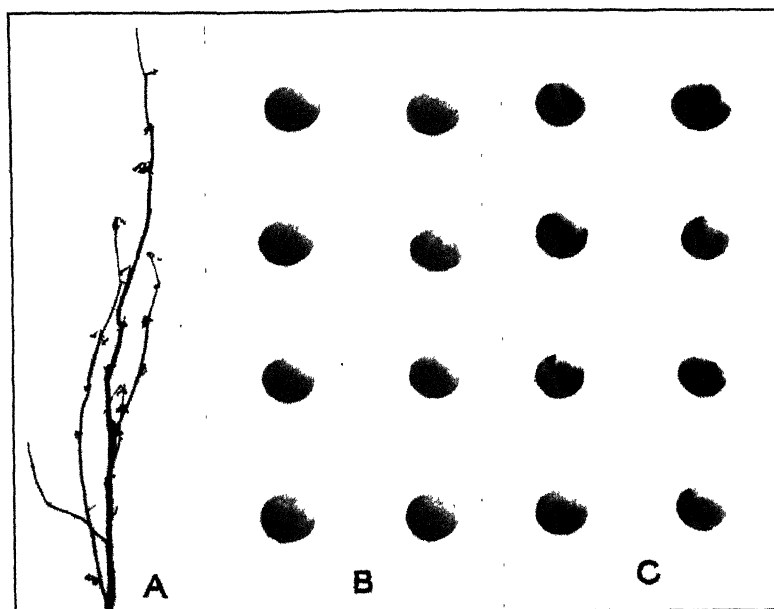
Fig. 1.—A, branch of an Ito San soy-bean plant bearing pure nonmottled seeds; B, typical seeds borne by the branch shown in A; C, typical seeds borne by the other branches of the plant. Plants of this type with branches bearing seeds entirely free from mottling when the rest of the plant bears mottled seeds are not common

In the fall of 1924 the writer became aware of the extreme variability of mottling found in seeds borne by the same plant. In an attempt to determine how general this variability is, several hundred plants were hurriedly examined in the field. In certain progenies, grown on certain types of soil, nearly every bean was mottled and the degree of mottling within the plant was fairly uniform. In other instances it was difficult to find a plant with a uniform lot of seed and occasional plants were found showing remarkable variation.

For a more detailed study, 25 plants showing some variation within the plant were selected, and a careful observation was made of the seed in relation to its position on the plant which bore it. Four plants were found having decidedly more mottled seed at the tip of the central stalk than elsewhere, but other plants showed the opposite tendency and, in general, a random distribution seemed most common.

In the fall of 1925 further studies were made of the distribution of mottled seeds on the plant, and from the evidence obtained it ap-

peared that mottling may in some cases be limited entirely to a single branch. Previously, the plants that were studied were threshed separately by means of a small machine. In 1925, however, the majority of the observations were made on pods picked at random from the plant. This method afforded ample opportunity to detect abnormal conditions, and six Ito Sans and three Mandarins were found which showed mottling only on a certain part. In these cases every seed on the branch was either pure yellow or mottled, and the mottling, where it occurred, was quite uniform (fig. 1).

DISTRIBUTION OF MOTTLED SEED WITHIN THE POD

In an effort to determine the relation between the occurrence of mottling and the position of the seed in the pod, 14 plants were examined, and each pod was classified. The results obtained are given in Table 1. In this study 2,186 seeds were observed, of which 1,165, or 53.3 ± 0.720 per cent, were mottled. The 1-seeded pods had 56.7 ± 2.18 per cent of mottled seed; the 2-seeded pods, 55.1 ± 1.18 per cent; and the 3-seeded pods, 51.3 ± 1.00 per cent. Although the 1-seeded pods had slightly more mottled seed than the 2 and 3 seeded pods, the difference is not statistically significant. The greatest difference is between the 2 and the 3 seeded pods, which is 3.8 ± 1.55 per cent, and this can hardly be considered significant.

TABLE 1.—*Relation between occurrences of mottling and position of seed in the pod in seed pods from 6 Mandarin, 5 Ito San, and 3 Manchu plants*

Position of seeds in pods	Mandarin						Ito San					Manchu			Total number of pods	Number of pods calculated on basis of random assortment
1-seeded pods:																
Pure yellow	4	7	4	13	6	10	3	6	0	12	7	12	13	5	102	110
Mottled	4	14	9	14	10	15	2	3	8	12	21	8	4	10	134	121
2-seeded pods:																
Both pure yellow	14	13	7	10	10	10	16	7	2	23	4	12	19	16	163	88
First, pure yellow; second, mottled	0	0	0	1	1	2	2	1	1	0	4	1	1	0	14	101
First, mottled; second, pure yellow	2	3	2	1	2	4	2	0	0	1	2	2	3	0	24	101
Both mottled	5	17	11	13	8	11	1	7	23	9	39	17	13	30	204	115
3-seeded pods:																
All pure yellow	15	5	2	2	2	5	18	56	0	15	0	9	7	13	149	58
First mottled, second and third, pure yellow	4	0	1	1	0	1	7	3	0	0	1	1	0	0	19	66
Second mottled; first and third, pure yellow	1	0	0	1	0	0	2	2	1	0	0	1	0	0	8	66
Third mottled; first and second, pure yellow	0	0	1	0	0	0	1	1	0	2	3	0	0	0	8	66
First and second mottled; third, pure yellow	0	1	1	0	0	1	4	2	3	0	4	1	0	0	17	76
First and third, mottled; second, pure yellow	1	0	0	0	0	1	3	1	0	0	1	1	0	0	8	76
Second and third, mottled; first, pure yellow	0	0	3	2	0	0	1	2	0	0	4	0	1	0	13	76
All mottled	3	11	5	2	3	4	6	7	56	4	19	12	8	18	158	86

Although the number of seeds in a pod does not seem to be a significant factor, it will be noted in Table 1 that in both the 2 and 3 seeded pods there is a strong tendency for the seeds in any one pod to be either all mottled or all nonmottled. If the probability of a seed being mottled is 0.533 and the probability of being nonmottled is 0.467, the probability of any combination can be readily calculated if mottling occurs at random. To illustrate the principle: A 3-seeded pod with the first seed mottled, the second nonmottled, and the third mottled, would have the probability of occurrence of 0.533 × - 0.467 × 0.533, that is, 0.133. The calculated results, assuming random assortment, are given in Table 1 for comparison with the observed.

The results given in Table 1 show that there is no particular position in the pod that is more likely to produce a mottled seed than any other position. In contrast with these results, Halsted (*3*) found that the position in the pod is important in determining the size of a seed. In both 2-seeded and 3-seeded pods he found that the basal seed was most likely to be the smallest and the seed at the tip of the pod the largest. In order to account for all of these results it is necessary to assume that mottling is not necessarily associated with the size of the seed.

Table 1 does not show the differences in degree of mottling, since every seed was classified as either pure yellow or mottled. Woodworth and Cole (*18*) go a step further and show that the type and degree of mottling within the pod is frequently uniform. The general conclusion can therefore be drawn that every pod is subjected to physiological conditions which affect the seeds within that pod in a similar manner. In plants that bear both mottled and nonmottled seed a very delicate balance must exist between the physiological conditions that cause mottling and those that prevent it. All the seeds on any one plant would surely be supplied with similar nutrients, and it is only when a very slight difference is significant that such striking contrasts should be produced as those exhibited by certain soy-bean plants.

ABNORMAL PHYSIOLOGICAL CONDITIONS

Mention has just been made of certain cases in which the mottled seeds were localized on certain branches, but in these cases the mottling was no more severe than is commonly found. On certain abnormal plants, however, the mottling was much worse than that which ordinarily occurs, showing the extreme effect which may be produced by environmental conditions. It is difficult to determine all the causes of this abnormal production of pigment, but the accumulation of synthetic products, especially sugars, seems the most probable underlying factor.

Plants with abnormal foliage have not been uncommon. Many developed crinkled leaves late in the season, but some exhibited an abnormal appearance from the seedling stage. In a few extreme cases the leaves were badly curled and spotted, presenting an appearance much like that of tobacco and potato plants infested with a mosaic virus. No success was attained, however, in transmitting the malady from one plant to another.

In the fall of 1924 one of these abnormal plants was found in a progeny of Manchu. This progeny had been selected for a uniform yellow seed coat and black hilum. It has since proved to be free from bad mottling, and subsequent progenies have bred true to type. The abnormal plant was very much stunted and none of the pods were normally filled out. It was about November 1 when the progeny was harvested, and at this time all the plants were perfectly mature except this abnormal individual. This plant alone was green, and although it had a sickly appearance it was undoubtedly manufacturing food material. The pods, however, had already turned yellow and had come to maturity. When these were opened the seed was found to be almost entirely self-black, exhibiting a very striking contrast to the pure yellow seed of sib plants. The most natural assumption was that this plant must have been a rogue. The black seed, therefore, was planted in 1925. The resulting plants produced seeds as free from mottling as those of the grandparent, showing conclusively that the plant with the black seeds was not a rogue and that the extreme type of mottling was due to physiological conditions rather than to heredity.

Other plants which appeared to be seriously diseased were found in strains of Ito San and Mandarin in 1924, but the degree of mottling did not seem to be markedly affected by the condition of the plant. In 1925, however, two Mandarin plants were found which had very much the same diseased appearance and their seeds were nearly self-brown.

In the fall of 1925 abnormal conditions were again observed, and these throw considerable light on the previous results and prove beyond all doubt that physiological factors may greatly influence pigment formation. Three Manchu plants were found on which some of the branches were broken over, and striking illustrations of variation of mottling within the same plant were obtained. The seeds on the broken branches were entirely normal, while those on the remaining central part of the plant were small and almost entirely black, presenting the same appearance as the seed of the abnormal Manchu plant found in 1924 which has just been described.

Figure 2 shows one of the abnormal plants as it grew in the field, and the contrast in the appearance of the seeds caused by the abnormal physiological conditions. That the branches had not recently been broken was evident from the nature of the breaks, yet all the pods on the broken branches were well filled out. The central part of each of the three plants appeared somewhat diseased; about half the pods failed to develop, and none were normally filled out. The leaves on the central stalk continued to grow after those on the broken branches had matured. It is very unlikely that a disease organism or virus was involved in bringing about the abnormal condition in the central stalks, for if such had been the case all the vascular tissue of the plant would have been affected.

Since the abnormal portion of these plants was green and was still manufacturing plant food, one would expect the amount of sugars as well as of other food materials to be increased. That the seeds were mature has already been mentioned, and the nature of the growth resembled very much that of sterile plants where no flowers are set and nothing but an accumulation of food material in the stems and leaves is possible.
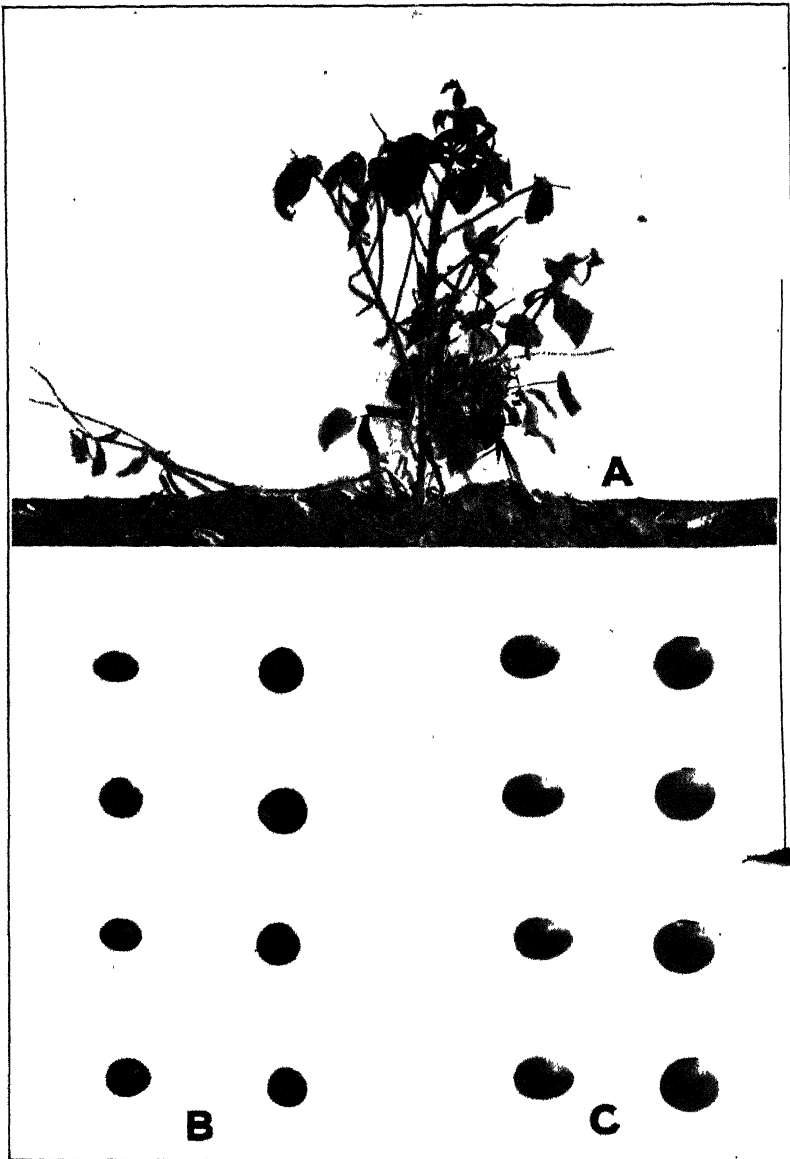
Fig. 2.—A, one of three abnormal Manchu plants of the same type found in 1925. The branches that were broken over produced normal seed while the remaining portion of the plant bore seed that was almost entirely black. B, typical seed from the central part of the plant. C, typical seed from the branch which was broken over

The reason why the pods matured before the leaves is not entirely clear. It is possible that the abnormal condition may have caused a deficiency of moisture and plant-food material earlier in the season and when the plant recovered and again resumed vigorous growth, the seed was physiologically mature and unable to make further growth. Moisture and food material supplied to these mature embryos at this time could result only in germination, as has been observed in the greenhouse when water is applied to mature plants. The nature of the enzymatic changes that take place in maturation make further growth impossible. The seed coat, however, is a tissue that easily absorbs moisture, so it seems entirely possible that an abnormal condition of this kind might markedly increase the concentration of sugars. If such an assumption is correct the excessive development of pigment in the seed coat might logically be expected.

Attempts have been made to produce abnormalities by thinning the pods on a plant in the expectation that the addition of food material which each seed would get would cause an increase in the quantity of pigment formed. Two Mandarin plants were treated in this way and expectations were fully realized. Figure 3 shows how the seeds from one of these plants increased in size and became nearly self-brown. On the other hand, many plants were treated in the same way and no effect on the amount of mottling was observed. There is room for a great deal more work along this line, but the results so far, although very poorly understood, are unique in demonstrating that mottling is not necessarily determined by the amount of food material present. Many very large seeds were produced by plants the pods of which were thinned without any apparent increase in the amount of pigment formation. The chemical balance of this food material therefore must also be important.

EFFECT OF LARGE AND SMALL QUANTITIES OF NITROGEN

Since a high relative amount of carbohydrates in comparison with nitrogen is known to affect the production of pigment in the leaves and stems of many plants [8] (*11*), it seems that the production of pigments in the seed coat should be similarly affected. If soy-bean plants could be grown with a high relative amount of nitrogen there should be little chance for carbohydrates to accumulate, with a possible corresponding absence of pigmentation.

With these facts in mind a project was undertaken in 1924 to determine, if possible, the effect of the carbohydrate-nitrogen relation on mottling. Two-gallon glazed pots filled with pure quartz sand were employed for this purpose, and the chemicals were applied according to the appearance of the plants. In this way plants were grown which were nearly yellow for lack of nitrogen and others were made very succulent by adding liberal quantities of nitrogen.

After ripening, the seeds from each plant were examined, but the data obtained were very conflicting. Some of the plants given only a very small amount of nitrogen produced mottled seed, and some given a large amount produced mottled seed. Plants bearing seed free from mottling were also produced under both conditions. The results indicate that the carbohydrate-nitrogen relation was probably not a factor in determining mottling, but the nature of the experiment

- [8] MacGillivray, J. H. effect of phosphorus on the nutrition of the tomato plant. 1925. [Unpublished doctor's thesis. Copy on file, library, Univ. Wisconsin, Madison.]
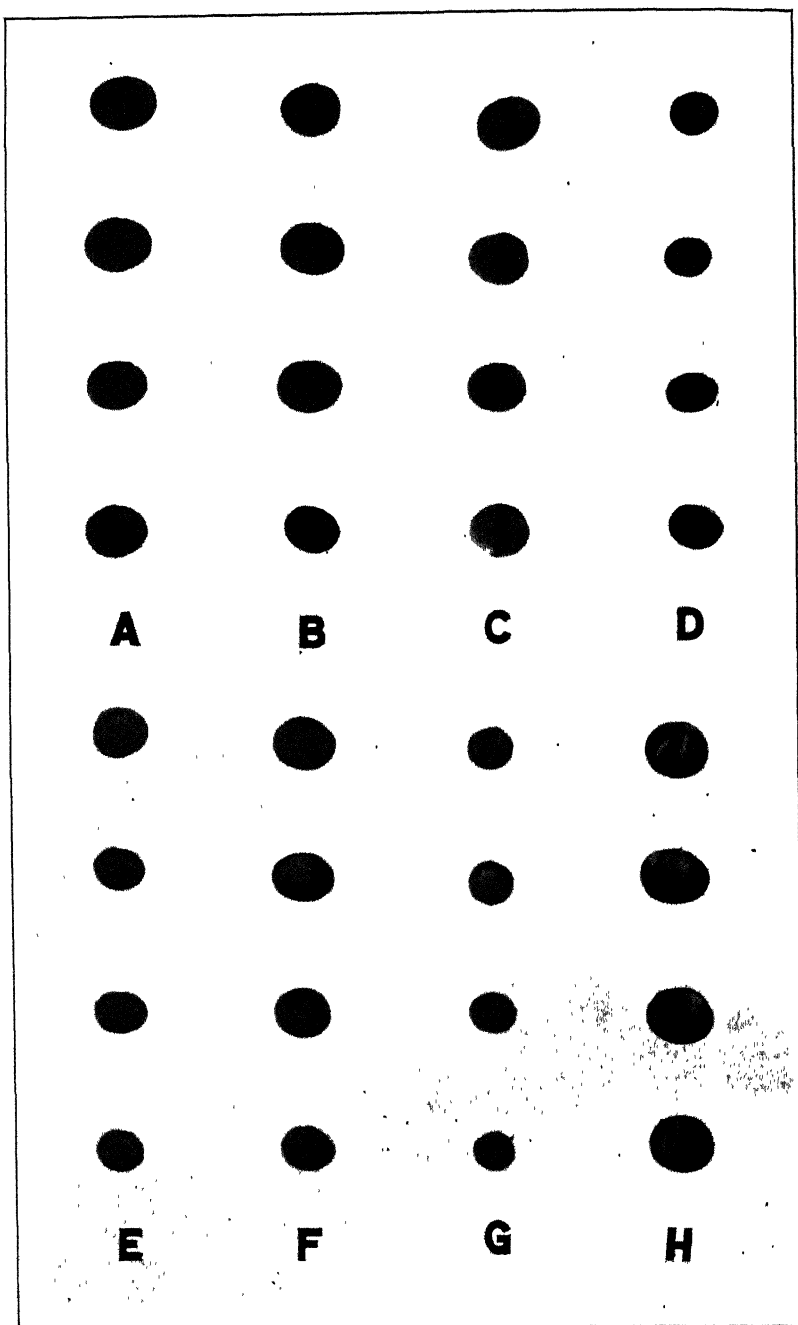
FIG. 3.—A to D, variation in mottling occurring in No. 2 strain of Manchu soy bean: A, seed from plant grown on sandy soil; B and C, seed from plant grown on rich soil; D, seed from an abnormal plant that presented a diseased appearance but that continued growth after the seed was mature. E to H, variation in mottling occuring in Mandarin variety: E, seed from plants grown close together in the row on a sandy type of soil at Spooner, Wis.; F, seed from plants grown on same type of soil as E, but spaced 3 feet apart in the row; G, small seed produced on uninoculated soil by plants that did not produce a thrifty growth; H, large badly mottled seed produced by thinning pods on a plant

does not warrant a definite conclusion on this point. It was very difficult to keep the plants growing uniformly, even those in the same pot, and this was particularly true as the time of maturity approached. All the nitrogen-high Mandarins, however, matured normally and very little mottling was noted on the seeds from these plants, but Ito San and Manchu plants grown under the same conditions were very much delayed in maturing and considerable mottled seed was produced. The time and nature of maturity, therefore, was probably a factor in the production of mottling, and it seems likely that it might have been more important than the condition of the plant in its earlier development.

TYPE OF SOIL AND DISTANCE BETWEEN PLANTS

In 1923 progeny tests were being run with Ito San and Mandarin seeds in cooperation with the United States Department of Agriculture. Three kinds of Ito San seed were planted, one of which had been breeding true for pure yellow seed. The other two lots had shown mottling, but in one case the mottled seeds were selected, and in the other case the nonmottled. Table 2 shows the behavior of these three selections when grown at Madison, Wis.

TABLE 2.—*Number of plants producing pure yellow and mottled seed in progenies from pure-line yellow, yellow, and mottled seed of Ito San soy bean, grown at Madison, Wis., in 1923*

Plot where grown	Seed planted	Number of plants producing pure yellow seed	Number of plants producing mottled seed	Per cent of plants producing mottled seed	Statistical comparison with pure-line yellow on Plot A			
					Difference in per cent	Probable error of difference in per cent	Difference ÷ probable error	Odds (approximate)
Plot A	Pure-line yellow	5	22	81.5				
	Yellow	6	23	79.3	−2.2	7.4	0.3	
	Mottled	2	20	90.9	+9.4	6.6	1.4	2:1
Plot B	Pure-line yellow	45	18	28.6	−52.9	7.4	7.1	435,000:1

On the same type of soil there was no significant advantage noted in the specially selected strain. A planting of the pure-yellow selection happened to be repeated on another plot, however, and an increase in mottling amounting to 52.9 per cent was obtained. This is over seven times the probable error of the difference to be expected from chance, and is surely significant.

Not only was there more mottling on one plot than on the other, but the degree of mottling was worse in plot A than in plot B (Table 2). It is not clear just what the difference in soil type was. A very thrifty growth was produced in both instances, and if anything, the plot producing the least mottling was the richer; but definite information is not available. The only point to be made is that mottling was affected by soil conditions.

During the summer of 1924 a study was made of the effect of soil-nutritional conditions. Five strains of seed were used in this project. Four single plants were chosen for four of the strains and a mixed sample was used for the fifth. One strain was selected from the pure-line Mandarins and another from the pure-line Ito Sans that

had been obtained from W. J. Morse, of the United States Depart-
ment of Agriculture. Two types of Manchu were grown, No. 1 and
No. 2. Strain No. 2 produced a larger plant and required a growing
period approximately two weeks longer than strain No. 1. The
mixed sample was from a pure line of Mandarins obtained from E. J.
Delwiche, of the Spooner, Wis., station. No mottling had pre-
viously been observed in this strain at the Spooner station, where the
selection was made.

One plot was selected on a sand hill where there was very little
chance for vigorous growth. The plants managed to survive and
grew to a height of about 10 to 12 inches, but very few seeds were set.
A second plot was selected on a rather heavy clay soil. Here the
plants grew to a height of 16 to 22 inches. The Mandarins seemed
to be particularly affected by this type of soil, for they were very
much smaller than the Ito Sans and Manchus, which was not the
case on the rich soil. For the third plot a rich loam soil that had been
heavily fertilized was chosen. Here all the plants grew vigorously
and the second strain of Manchus reached a height of 28 inches.
On the second and third plots the plants were all spaced 3 feet apart,
but on the sandy soil they were planted only 1 foot apart because of
lack of space.

TABLE 3.—*Effect of soil type and thickness of stand on mottling in soy-bean seed*

[Summary of results obtained in 1924 with Mandarin, Ito San, Manchu No. 1, and Manchu No. 2]

Type of soil and distance apart of plants in row	Height of plants in inches (approximate)	Total plants threshed	Per cent mottled [1]	Per cent medium mottled or worse
Light sandy soil; plants 1 foot apart	10–12	66	6	1
Heavy, rather infertile clay soil; plants 3 feet apart	16–22	226	66	31
Rich loam soil; plants 3 feet apart	24–28	125	100	90
Rich loam soil; plants approximately 1 inch apart	40–50	161	26	12

[1] Individual plants were arbitrarily classified as nonmottled, slightly mottled, medium mottled, and
badly mottled; so the percentages refer to the number of plants rather than to the total number of seeds.

It will be noted in Table 3 that practically all the plants on the
sandy soil produced pure yellow seed. Mottling occurred on the
plants grown on the heavy clay soil, but most of it was rather faint,
especially in the case of the Mandarins. On the rich soil, however,
every plant produced mottled seed, and in 90 per cent of these cases
the mottling was bad.

At the time that these observations were made varietal tests were
in progress with certain Ito San, Mandarin, and Manchu selections
growing on rich soil not far from the plot where mottling occurred in
such abundance. These varieties were grown from seed very closely
related to that which was used in the mottling project; so a comparison
is not out of place. In the varietal tests the plants were close to-
gether in rows 3 feet apart and the rate of seeding was 1 bushel per
acre. A very different type of plant from one grown 3 feet apart was
produced under such conditions. The stems were very much thinner
and the height of the plants was 40 to 50 inches, or nearly twice that
of the plants spaced 3 feet apart. The growth was in many ways

similar to that obtained by growing plants in the greenhouse under reduced light. The number of seeds per plant was greatly decreased and the seeds themselves were much smaller.

Table 3 gives the combined results for the Ito San, Mandarin, and Manchu varieties spaced 1 inch apart. Eighty-eight per cent of these plants bore seed practically free from mottling, whereas a very similar soil failed to produce a single plant that did not show mottling when spaced 3 feet apart. The matter of thickness of stand, as well as the type of soil, therefore, appears to be an important factor in the production of mottling.

In 1925 an attempt was made to repeat the work done in 1924, and to get additional information in regard to the effect on mottling in seeds from plants grown on different types of soil with different distances between them. Table 4 gives some of the results. The original strains of Ito San and Manchu were used in this project, and a selection of Mandarins was made from the pure line obtained from the Spooner station. In addition to observations on selected plots of different types of soil at Madison, a test was run at the Spooner station, where mottling had never previously been observed to any great extent.

TABLE 4.—*Comparison of amount of mottling produced on seeds of different varieties of soy beans when plants are spaced at different distances on various types of soil; 1925 experiments*

Description of soil	Distance apart in row	Variety	No mottling (pure yellow)	Slight mottling	Medium mottling	Bad mottling	Total plants observed	Per cent mottled
Rich loam, Madison, Wis. (inoculated).	3 feet	Mandarin	3	9	12	0	24	88
		Ito San	0	0	0	25	25	100
		Manchu No. 1	10	4	5	0	19	47
		Manchu No. 2	3	10	3	1	17	82
	4 inches	Mandarin	3	6	2	0	11	73
		Ito San	0	0	0	25	25	100
		Manchu No. 1	19	0	0	0	19	0
		Manchu No. 2	2	6	3	0	11	82
Rich loam, virgin to soybeans, Madison, Wis. (not inoculated).	3 feet	Mandarin	5	8	5	0	18	72
		Ito San	0	0	0	50	50	100
		Manchu No. 1	30	0	0	0	30	0
		Manchu No. 2	2	11	30	5	48	96
	1 inch	Mandarin	10	19	17	0	46	78
		Ito San	5	5	26	4	40	88
		Manchu No. 1	25	0	0	0	25	100
		Manchu No. 2	4	7	23	4	38	89
Light and sandy, Madison, Wis. (inoculated).	3 feet	Ito San	11	0	6	0	17	35
		Manchu No. 1	33	0	0	0	33	0
		Manchu No. 2	12	1	3	0	16	25
	1 inch	Ito San	0	6	20	0	26	100
		Manchu No. 1	57	6	0	0	63	10
		Manchu No. 2	0	7	18	0	25	100
Light and sandy but in high state of fertility, Spooner, Wis. (inoculated).	3 feet	Mandarin	6	9	5	0	20	70
		Ito San	0	0	0	25	25	100
		Manchu No. 1	23	0	0	0	23	0
	1 inch	Mandarin	100	0	0	0	100	0
		Ito San	53	0	0	0	53	0
		Manchu No. 1	25	0	0	0	25	0

From Table 4 it is apparent that the plants on sandy soil again showed a tendency to produce seed free from mottling, regardless of whether they were spaced 1 inch or 3 feet apart. From August 8 to September 6 there was only 0.16 inch of rainfall at Madison, and

the effect on the soy-bean plants grown on the sandy soil was notice-
able. After September 6 when rains again fell certain plants began
to take on a vigorous growth. This growth affected the plants in
such a way that there were marked differences in the time of matu-
rity. The plants spaced 3 feet apart set many more seeds, survived
the drought in a more normal condition, and matured earlier than
those that were spaced only an inch apart. This was particularly
apparent on the sandy soil in the case of Ito San, Mandarin, and
Manchu No. 2.

The first strain of Manchus, as already mentioned, matures earlier
than the second. This was especially noticeable on the sandy soil
at Madison where the first strain was too nearly mature to be revived
by the September rains, and the seed was entirely free from mottling.
The Mandarins were more affected by the drought than the other
varieties. In most cases less than six seeds were set per plant and
some plants failed to set any, but after the heavy rains in September
all these plants took on a dark green color and began to grow. The
seeds produced, while few in number, were even larger than normal
seed and were badly mottled. The Mandarins on the sandy plot
produced so small a quantity of seed that the data for this variety
are not given in Table 4. Very similar results, however, were obtained
in the case of Ito San and the No. 2 strain of Manchu.

Unless all the conditions of the experiment are considered, a hazy
idea is likely to be obtained from Table 4 as to the effect on the
plants of the difference in distance between them. It is evident
that spacing is a factor of importance only in certain cases. On the
rich soil all four strains mottled so badly that no difference resulting
from spacing was noticeable except in the Manchu No. 1 strain.
Here the seeds from 47 per cent of the plants 3 feet apart produced
mottling, while all seeds from those grown 1 inch apart were pure
yellow. The opposite condition was observed on the sandy soil, as
already explained, plants spaced 3 feet apart producing the purer
seed.

The most striking effect of spacing was noted at the Spooner station.
Seeds of the first strain of Manchu were pure yellow even when
planted 3 feet apart; but all the Ito Sans and Mandarins grown 3 feet
apart tended to be badly mottled; while the Ito Sans and Mandarins
closely spaced, in rows grown for varietal trials, produced seed entirely
free from mottling. It should be mentioned that the type of soil at
the Spooner station was a light sandy loam but well fertilized. Since
mottling appeared in a marked degree on the Ito San and Mandarin
varieties when spaced 3 feet apart, it may be assumed that the reason
why mottling had never been observed there before was because all
observations had been made on plants grown close together for
varietal trials.

Table 4 also shows the results obtained from growing the same
varieties on a soil virgin to soy beans without inoculation, all other
plots being inoculated. The plants on this plot were not particularly
thrifty, yet mottling was very prevalent in all varieties except the
No. 1 strain of Manchu. Plants grown on this plot were so poorly
nourished that the seed was often very small, but at the same time it
was badly mottled. In previous instances mottling seemed more
likely to be associated with large seed produced on rich soil. This
point is of particular interest because it shows again that the total

amount of nutrient material may not be the determining factor in mottling. The seeds produced by the same strain on the sandy soil were well filled out in many cases and at the same time were free from mottling.

TABLE 5.—*Effect of thickness of stand on mottling of seed in a commercial variety of Manchu soy bean*

Distance apart of plants in row	Number of plants producing seed that showed—				Total	Per cent mottled
	No mottling (pure yellow)	Slight mottling	Medium mottling	Bad mottling		
3 feet	34	14	18	1	67	49
1 inch	160	3	0	0	163	1.8

Table 5 shows better than Table 4 the effect on mottling of the distance between plants in the row. In the experiment reported in this table an ordinary variety of Manchu was used in which there may have been unrelated strains, but the numbers are large enough to give a good random sample in each case. Two rows of the ordinary thick planting used in varietal trials were simply thinned so the plants were 3 feet apart in the row. The table shows that seed from plants grown close together was much freer from mottling than seed from plants grown farther apart.

FERTILIZERS

Additional work was carried on in 1925 to determine the effect which fertilizers might have on mottling. On the rich soil at Madison a row of each of the four strains used in the other trials was treated with a heavy application of sodium nitrate. A test was also run on peat soil with applications of phosphorus and potassium to determine the effect of these fertilizers on a soil in which they are supposedly lacking. Most of the plants produced on these soils bore mottled seed, but there was no apparent effect from the fertilizers in any case. The fact that the results were negative does not prove, however, that these fertilizers may not be important factors in producing mottling; but simply that they appeared to exert no influence in these particular cases.

INOCULATION

Insufficient work has been done to allow definite conclusions to be drawn as to the effect of inoculation on mottling, but there is good reason to believe that it is an important factor. On rich soils where mottling has been pronounced, nodule production has been poor, and on sandy soils where little mottling has been observed nodule production has been exceptionally good.

No satisfactory explanation has been given as to why there is poor nodule production on certain types of soil, yet considerable difficulty has been experienced in growing soy beans because of this fact. Perhaps the most illuminating work in this field is that concerned with the behavior of different strains of nodule-producing bacteria. Wright (*19, 20*) has studied and described two types of *Pseudomonas*

radicicola, the bacterial organism causing nitrogen-fixation in soy beans. Three years' work showed that plants inoculated with type A fixed much more nitrogen than plants inoculated with type B.

A possible explanation may be given, therefore, of a very common observation, that is, in cases where no mottling occurs the first year soy beans are grown, in later years it may become very pronounced. If a poor nitrogen-fixing strain of bacteria should gain preponderance over the original good nitrogen-fixing strains, one would naturally expect mottling to be affected.

SHADING

In 1923 approximately 300 plants, including all the strains that were being tested for the inheritance of mottling, were bagged to insure self-pollination. After the pods had set and the bags were removed, it was apparent that the shading had caused the stems to be finer in texture, but that of all the 300 plants only 4 were free from mottling. Very similar results were obtained in 1924, about the same number of plants having been bagged. Hollowell's[9] observations in regard to shading accord with these results.

Although bagging in the field did not prevent mottling, very different results were obtained by growing plants in the greenhouse. In the winter of 1923-24 a number of varieties, together with some hybrids, were grown in the greenhouse with the aid of artificial light. A genetic study was the chief object of these experiments, but the observations on mottling are of interest as well. Approximately 250 plants were grown and not the slightest sign of mottling was noted in any of the seeds produced. The same number of plants were grown in 1924-25 with almost the same results. It is difficult to explain these findings, but since the plants produced a much more spindling growth one would expect that light intensity was an important factor.

To get more information regarding light intensity, six plants from each of the four strains used in the mottling test in 1925 were grown under a frame covered with three thicknesses of cheesecloth. The plants grew normally under these conditions, the only noticeable differences being a tendency to grow higher and to produce slightly weaker stems. All of these plants produced mottled seed, and although the amount of mottling may have been slightly less than in the controls, the difference was not significant.

Although it seems logical that shading should be an influencing factor under certain conditions, all attempts to reduce the amount of mottling by shading in the field have failed. Of course, the length of the light wave as well as the intensity of the light may influence mottling, but this factor has not been taken into account. Since a large percentage of the ultra-violet light is screened out by ordinary glass it may be possible that mottling did not occur in the greenhouse for this reason; but studies under controlled conditions are necessary before any proof can be established.

[9] HOLLOWELL, E. A., FACTORS INFLUENCING THE MOTTLING OF THE SOY BEAN SEED COAT. 1924. [Unpublished master's thesis. Copy on file, library, Iowa State College, Ames.]

HEREDITY IN RELATION TO MOTTLING

Attention has been called to the wide variation in mottling, which may be caused by environmental conditions. It has been proved that these variations may occur in the same pure-line selections independent of hereditary influences, but the differences which have been observed among different varieties and among selections of the same variety show that there is also a tendency for mottling to be inherited.

During the course of this study considerable effort has been made to select for and against mottling. These efforts have met with remarkable success, but not within related progenies. When once a selection was made the tendency was to breed true except for the variation that could be accounted for as due to environmental influences. One selection alone (No. 83 in the Black Eyebrow variety) showed signs of segregation. The evidence indicates that the original seed selected in this case was heterozygous, but all the other numerous selections were apparently homozygous to start with. For this reason mottling can not be attributed to hybridization or to mutations, at least not the large amount that is commonly observed. This does not mean, however, that mutations or hybridization may not occasionally occur, but it is unlikely that they occur with very great frequency.

Genetic analyses (*13*) revealed the important fact that the self-color type in soy beans is a recessive character and that a multiple series of factors is responsible for preventing the development of pigment in the seed coat. I^h is the factor which tends to inhibit all pigment fomation, I^1 inhibits pigments to the hilum, I^k inhibits them in such a way as to form an "eyebrow" pattern, as in the Black Eyebrow variety (fig. 4), and i is the recessive factor which has no effect. With an allelomorphic series of this sort all gradations of restriction factors may be imagined, and this seems to be the logical interpretation of the results which have been obtained. Mutations within such an allelomorphic series would not be unexpected and this would provide a method by which heritable differences may occasionally be produced.

Although these restriction factors (I^h, I^1, and I^k) have been spoken of as dominant, it should not be implied that they are fully dominant. The relationship of I^k to i has not been established, but I^h and I^1 are only partially dominant over i. Therefore, hybridization with a self-colored variety increases the mottling in the F_1 plant (fig. 4).

Besides this allelomorphic series which inhibits the development of pigment, there are other factors that may be considered as modifying its development. The most important of those which have been identified is the factor T, for tawny pubescence. This factor affects both the quality and quantity of pigment formed. It is possible that there are other factors in the same class and the relationship may be very indirect. Since mottling is known to be easily influenced by any change in the nutritional balance of the plant, there is considerable opportunity for factors primarily concerned with the physiological behavior of the plant to have a modifying effect on mottling.

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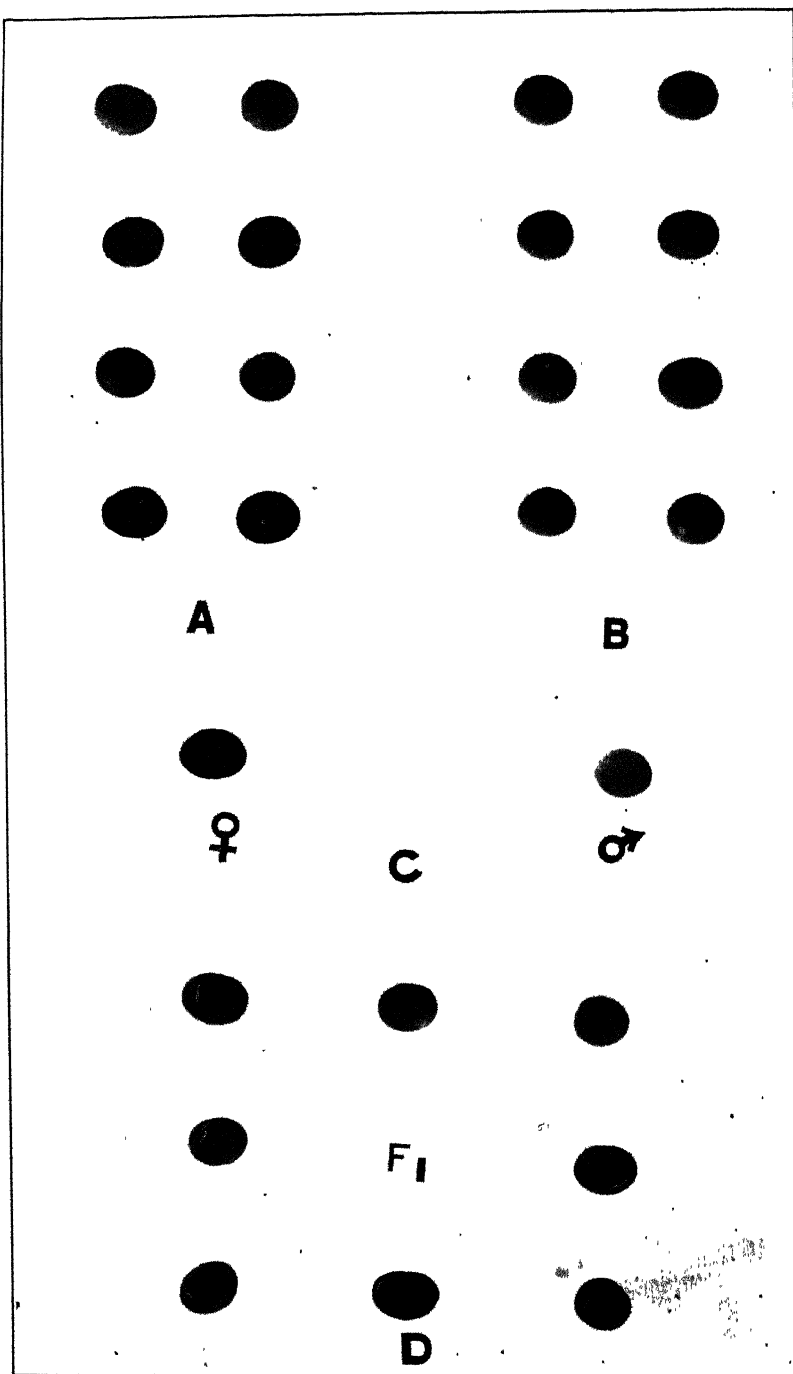
Fig. 4.—Differences found to be due to heredity in the "eyed" or "patched" pattern of the Black Eyebrow variety of soy bean: A, nearly black seeds typical of strain No. 82; B, seeds with typical uniform pattern from strain No. 84. C^1, ♀, a black seed from a selection of an unknown variety; ♂, a Mandarin; D, F_1 seeds, showing some of the variations in mottling found in the hybrid plant

From the limited amount of work so far accomplished it is difficult to distinguish the influence of heredity from that of environment. Environmental influences are so strong that it is impossible to form any judgment of the hereditary potentialities by the appearance of the seed from any one plant. The only safe method is to grow a number of seeds from the plant in question and to make statistical comparisons with other progenies for the amount of mottling produced. This is necessarily slow work and the number of progenies that can be tested is limited.

SELECTING AGAINST MOTTLING

In 1922, 206 plants were grown from seed selected for different types of mottling, and 94 of these were bagged with cheesecloth to prevent cross-pollination. Most of the plants grown from this seed were badly mottled and only 10 were free from mottling. The type of mottling varied greatly, only 4 plants breeding true for the particular pattern of the parental plant.

In 1923 a further attempt was made to study the inheritance of mottling in these selections. As a general rule the mottling had been so pronounced that an effort was made to select out nonmottled strains. In 1922 certain progenies of Manchu had seemed to be much more susceptible to mottling than others. From the seed produced eight progenies were grown for comparison with other selections which showed a tendency to be free from mottling. Table 6 gives the results of these experiments. Considerable variation was noted within each of these progenies, but Table 6 shows that there was much more mottling in the eight selections than in the other progenies. This means that there must have been heritable differences, but each selection seemed to breed approximately true to the original type. Since there were no signs of segregation, it is logical, in spite of evidence of heritable differences, to assume that the original selections were homozygous and that the variations which occurred within each selection were due to environment.

TABLE 6.—*Degree of mottling apparent in progenies from unmottled seed of low-mottled strains, mottled seed of low-mottled strains, and mottled seed of high-mottled strains of Manchu soy beans; 1923 experiments*

Parental stock	Number of progenies grown	Number of plants with seeds that showed not more than a trace of mottling	Number of plants with seeds that showed slight mottling	Number of plants with seeds that showed medium or bad mottling
Nonmottled seed from low-mottled strains	23	153	54	32
Mottled seed from low-mottled strains	15	63	26	10
Mottled seed from high-mottled strains [1]	8	0	7	120

[1] The plants from the high-mottled strains which were selected for this study were comparable to the more badly mottled plants from the low-mottled strains.

In 1924 two strains of Manchu were selected for study, No. 1 and No. 2. Strain 1 was originally nearly free from mottling and throughout all the tests that were made in 1924 and 1925 it continued to breed true, except under environmental conditions most favorable

for mottling. Strain 2, however, which was originally badly mottled, constantly produced bad mottling except under environmental conditions unfavorable for the development of mottling.

Selection within varieties other than Manchu has been attempted, but no success has been attained. In 1923, 43 progenies were grown from plants which had produced brown mottling. Ten of these were probably Ito Sans and one was evidently a selection from the Aksarben variety; the other 32 progenies were not identified as belonging to any particular variety.

Only three plants from the Aksarben strain matured in 1923. These plants produced pure yellow seed, but in 1924 and 1925 all Aksarben progenies were mottled. The Ito San progenies in 1923 also showed some freedom from mottling and two plants bore pure yellow seed. From Table 7 it is apparent that environmental influences must have been responsible for the fact that these plants produced pure yellow seed, because in 1924 their offspring were just as badly mottled as the progenies from plants showing bad mottling in 1923. The 32 progenies grown in 1923 which could not be identified with any particular variety, continued to produce mottling in 1924. There was often considerable variation from one plant to another in the same progeny, but this was to be expected since environmental conditions were not entirely uniform.

TABLE 7.—*Number of plants producing mottled seed in progenies grown from (1) medium to badly mottled seed derived from strains showing some freedom from mottling, (2) medium to badly mottled seed derived from strains showing no freedom from mottling, and (3) seed free from mottling; Ito San variety of soy bean*

Parental stock	Number of progenies grown	Number of plants producing seed that showed medium or bad mottling	Per cent of plants producing mottled seed
Medium to badly mottled seed, from strains showing some freedom from mottling	4	98	100
Medium to badly mottled seed, from strains showing no freedom from mottling	4	56	100
Yellow seed free from mottling	2	33	100

These results show that it has been possible to select for strains that are more inclined to mottle than others. The seed from which selections were made, however, may have been unrelated, so it can not be said that there has been successful selection within any one related line. The original seed in these cases must have been homozygous. If heterozygous material had been selected instead of homozygous it is entirely possible that selections could have been made within individual progenies. If mottling were produced by frequent somatic mutations this should also have made it possible to select for or against mottling.

SELECTING FOR PIGMENTATION PATTERNS

A random assortment of seed from the Black Eyebrow variety was planted in 1922 and six plants, Nos. 81 to 86, were grown to maturity and threshed. These plants were spaced 3 feet apart and were

bagged with cheese cloth during the flowering period. After thresh-ing, considerable variation was observed among the six plants, but No. 84 was different from the rest in that its mottling pattern was reasonably uniform and somewhat lighter.

A progeny from each of the first four plants was grown in the same manner in 1923 except that it was impossible to bag every plant. After threshing and classifying according to the uniformity of the pigment pattern, 17 plants grown from No. 84 were found to have regular and uniform patterns like that of the parent. In contrast with this light uniform pattern, 17 plants from No. 82 produced seed of which much was entirely black.

Nine plants from No. 81 and nine from No. 83 showed considerable variability. Eleven of these plants showed a reasonably uniform pattern while the remaining seven produced very dark-colored seed. Figure 4, A and B, illustrates the difference in appearance bet een the uniform pattern of No. 84 and the dark pattern of No. 82.

TABLE 8.—*Number of plants in progenies of Black Eyebrow soy beans producing seeds with typical eyebrow pattern, pattern slightly extended, mostly obliterated, and quite obliterated*

Parental stock	Number of progenies grown	Number of plants producing seed of—			
		Typical eyebrow pattern [1]	Pattern slightly extended	Pattern mostly obliterated	Pattern quite obliterated (seed nearly black)
Plants with uniform patterns, from No. 84	5	271	7	0	0
Plants with uniform patterns, from No. 83	9	212	138	13	0
Plants with dark seed, from No. 83	4	0	6	122	12
Plants with variations in patterns, from No. 82	2	0	0	51	2
Plants with very dark seed, from No. 82	4	0	0	105	61

[1] See Figure 4.

The next season (1924) 2 progenies from No. 82, 8 from No. 83, and 2 from No. 84 were tested. Table 8 gives the results of these experiments as well as the data from further work in 1925. That these three strains of Black Eyebrow had different hereditary poten-tialities for the development of a mottling pattern is evident from the table. All of the 278 plants from No. 84 showed a high degree of uniformity, but those from No. 82 continued to produce dark seed, and not a single plant of the 219 grown from No. 82 was com-parable in pattern type with the No. 84 strain.

The behavior of progenies from No. 83 indicates very strongly that the original seed selected was heterozygous, but it is the only example of successful selection toward a particular mottling pattern that the writer has observed. None of the progenies from No. 83 produced seeds quite as uniform as those from No. 84, but some were practically as dark as those from No. 82. Three progenies from No. 83, selected for a uniform pattern, continued to produce a uniform pattern in 1925 and two progenies from the dark-seeded plants con-tinued to produce dark seeds.

Although it seems logical to assume that No. 83 was originally heterozygous, it would be useless to theorize on the factorial relationships because of the nature of the variability and the limited number of progenies grown. Heredity is undoubtedly important in producing different amounts of pigment but all other selections happened to be made from homozygous material that was no longer amenable to selection. This example alone affords proof of successful selection within a related strain, and the fact that No. 82 and No. 84 remained constantly different is also proof of hereditary differences.

RELATION BETWEEN COLOR OF PUBESCENCE AND MOTTLING

Among the varieties subject to brown mottling there are noticeable differences in shades and intensities. The stage of maturity and abnormal weather conditions at harvest time may influence the color, but there are very constant differences between certain varieties. Nagai (8) has called attention to some of these differences and has determined the genetic behavior of certain browns, buffs, and reddish browns.

In the determination of the shade of brown pigment, the color of pubescence is important. Mandarins, Aksarbens, and certain Chinese varieties that have been grown have a gray pubescence and produce a very light-brown mottling. This light-brown color is the "ochraceous tawny" of Ridgway (15). Ito Sans, however, have a tawny pubescence, and the mottling color "auburn" is much more intense than that produced by gray-pubescent varieties.

Not only is the mottling darker on varieties with tawny pubescence but the tendency to produce mottling seems to be more pronounced, at least on certain types of soil. Experiments have shown that Ito Sans are usually more likely to produce mottled seeds than Mandarins. The gray-pubescent varieties other than Mandarin have not been so extensively studied, but they also seem to be freer from mottling than varieties with tawny pubescence.

A hybrid between an Ito San and Aksarben is of interest in this connection. The F_1 plant had tawny pubescence like the Ito San parent, but it was grown in the greenhouse where only a trace of mottling developed. In the F_2 population 59 plants were grown and a classification based on color of pubescence and degree of mottling gave the following results:

	No mottling	Slight mottling	Medium mottling	Bad mottling
Tawny-pubescent plants	0	20	19	9
Gray-pubescent plants	5	4	2	0

Another cross was made between a self-colored brown with tawny pubescence and a Mandarin. In the F_2 generation tawny and gray-pubescent plants occurred in a 3:1 ratio. Plants with mottled and self-brown seed also occurred in a 3:1 ratio, making a 9:3:3:1 ratio when all characters were considered. All the gray-pubescent plants again showed the light-brown pigmentation, and tawny pubescence was associated not only with a darker brown pigment but also with a greater degree of mottling. The results obtained were as follows:

	Slight mottling	Medium mottling	Self-brown
awny-pubescent plants	5	35	10
ray-pubescent plants	9	1	6

It is not known whether the pigment in Mandarin seed coats is different from that of tawny-pubescent varieties, but abnormal Mandarin plants have been referred to which have developed a pigment nearly as dark as any brown variety. It seems entirely possible therefore that the difference in color may be due to the fact that the pigment is more concentrated in the seed coats of the tawny-pubescent plants. For this reason it seems logical that the factor *T*, for tawny pubescence (*13*), affects not only the shade of color but also the quantity of pigment formed.

Attention has already been called to the fact that Mandarin is more sensitive to certain types of environment than are some other varieties; so it can not be assumed that results similar to those just described would always be obtained. In some instances Mandarin has produced just as much mottling as Ito San or any other tawny-pubescent variety.

CROSS-FERTILIZATION

Piper and Morse (*14*) have pointed out that mottling may result from hybridization. All the crosses made by the writer between mottled and self-colored varieties confirm this statement. Self-colored black or self-colored brown seeds invariably have been recessive to yellow or green. A single pair of factors has been involved in all cases, but the dominance has been noticeably incomplete.

Figure 4, D, shows the typical coloration in the seed coats of an F_1 plant from a black × Mandarin cross. The black pigment was dominant to the brown carried by the Mandarin, but the seeds borne by the F_1 plant were generally badly mottled with black. This mottling was much more extensive than the brown ordinarily produced by Mandarins.

In an F_2 population from a cross such as the one just described, plants were produced with mottled or self-colored seed coats and the pigment was either black or brown, depending on the presence of R_1, the factor for anthocyanin pigment. Among the mottled seeds such striking variations in the amount of mottling have been observed that they can hardly be accounted for by environmental conditions.

Further study is necessary to explain these extreme variations in F_2 populations. It is possible that modifying factors may affect the expression of the single inhibiting factor. But as already mentioned, a heterozygous plant of the type resulting from a yellow × black cross is usually more mottled than the yellow parental variety. This complicates the situation in an F_2 population because it is possible that the plants with very badly mottled seed may simply be heterozygous for a single factor.

EXTENT OF NATURAL HYBRIDIZATION

In examining plants for mottling, the writer has occasionally chanced on a natural hybrid. During the last three years six plants have been found that might be classed as such, but in the course of

these observations over 20,000 plants have been examined; so the actual percentage of natural hybridization, although it occurs, is surely very low.

Woodworth (*17*), in a specially planned experiment, found that cross-pollination between, two soy-bean varieties growing close together was approximately 0.16 per cent, but a somewhat higher percentage has been reported by Piper and Morse (*14*). The writer has not carried on a test comparable to Woodworth's, but from 15 plants with green cotyledons growing 3 feet from a variety with yellow cotyledons 5,500 seeds were obtained, 6 of which had yellow cotyledons, showing that they were the result of hybridization. This means that 0.11 per cent of the ovules had been cross-fertilized by pollen-carrying factors for yellow cotyledons; multiplying this figure by four to allow for the other possible combinations which would not be observed, gives 0.44 per cent.

The writer has not observed that honeybees or wild bees visit soy-bean flowers with any great frequency. Moreover, practically all of the flowers are fertilized before opening. This being the case, thrips are the most likely agency for the small amount of crossing that has been observed. Several species of thrips have been seen in soy-bean flowers and sometimes they are very numerous at flowering time. Frequently they have been seen making their way through the corollas of flowers before the pollen is ripe.

DISCUSSION

From the data presented in this paper, as well as from the results obtained by Hollowell [10] in Iowa, it is apparent that mottling is in some cases due to environmental factors. As a rule plants grown on a rich soil and spaced 3 feet apart produce badly mottled seed. Certain physiological abnormalities, however, have been responsible for the most extreme development of pigment.

Large seeds are produced on rich soil when plants are spaced 3 feet apart, but many cases have been cited where mottling has been associated with very small seed. The plants grown on a low-nitrogen nutrient solution furnish a striking example of this coincidence. Therefore, the accumulation of a large amount of synthetic material by the plant can not be the sole cause of mottling. On sandy soil thrifty plants have been grown, and large seeds entirely free from mottling have been produced.

It seems possible that the maturation of the seed may be a very important factor in the production of mottling. As long as the seed is growing a natural place is provided for the accumulation of synthetic material; but anything that causes premature maturation of the embryo, as when certain abnormal physiological conditions prevail, may bring about an accumulation of food material in other parts of the plant. Since mature cotyledons could not make use of these food materials, there would be an accumulation somewhere if the plant persisted in the process of photosynthesis. An accumulation in the seed coat under these conditions would explain how mottling may be brought about, and the development of pigment due to this cause corresponds very closely with the development of pigment by decortication, because in each case there is an accumulation of synthetic material in the tissue where the pigment is formed.
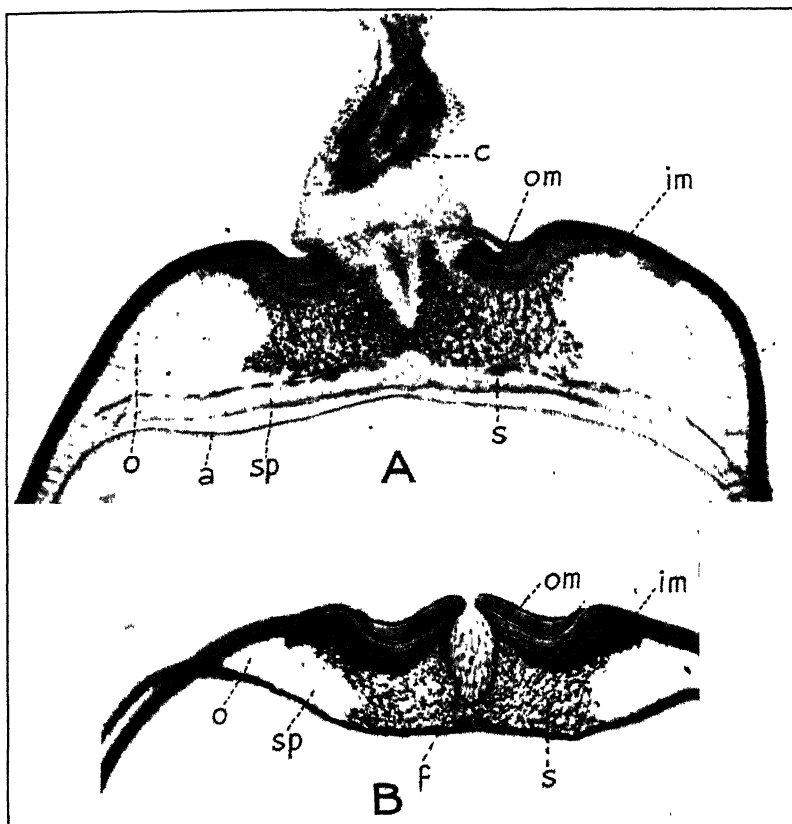
Cross sections of hilum of soy beans, showing how the pigment is distributed in the seed coat. There was no staining, so all coloration is due to the natural pigments.
A, cross section of hilum from seed produced by hybridization with the Soysota variety.
B, cross section of hilum from the Wisconsin Black variety.   This is the normal type of hilum-for most varieties and it is dominant over the above type in inheritance: im, inner Malpighian cells; om, outer Malpighian cells; o, osteosclerid cells; c, remains of funiculus; sp, spongy parenchyma; s, asteroid parenchyma; f, fibrovascular bundle of testa; a, aleurone layer.

The study of mottling in relation to the position on the plant is particularly illuminating because it indicates that pigment formation may be controlled by a very delicate balance in nutritional conditions. The fact that seeds in the same pod always tend to be alike in spite of a wide variation within the plant emphasizes the importance of this very delicate balance.

The part played by restriction factors is not entirely clear, although observations indicate the presence of an enzyme or some active principle which inhibits the development of pigment. If such an enzyme is constantly at work preventing the formation of mottling pigments, any addition of sugars, the raw material from which these pigments are probably formed, would give this restriction mechanism an extra task. Considering the problem in this light, the whole question of mottling may well be reversed. Instead of inquiring about the factors responsible for mottling, why not ask, What are the inhibiting elements that prevent pigment formation?

Some strains of soy beans consistently produce more mottled seed than others, but the relative difference between two varieties may not always be the same. It has been found that a particular environment may affect some varieties differently. The Mandarin variety is not adapted to poor soils, especially heavy clays. Since the whole growth of the plant is more markedly affected by this kind of environment than are some other varieties, it is not surprising that mottling should also be affected to a greater extent. Such has actually been found to be the case. In 1924 the Mandarin plants grown on heavy clay soil were much more free from mottling than Manchus and Ito Sans grown on the same soil, a difference not so noticeable on richer soil.

In behavior, mottling is very much like the production of anthocyanin pigments in many other plants. Numerous factors may be involved, but the most important factor in the development of anthocyanin pigments is always heredity. Hereditary factors may entirely control the formation of pigment if the plant succeeds in growing at all. In other cases hereditary factors simply make it possible for the pigment to develop; and still other instances are known where no pigment ever develops, corresponding to albinism in animals. Borrowing the term "albinism" with this shade of meaning, we might say that there are no soy beans with "albino" seed coats as far as is known. All varieties apparently have the power to develop a certain amount of mottling under the proper conditions.

Where heredity alone is the controlling factor, other factors conducive to pigment formation, although still active, are obscured. Black and brown varieties of soy beans have such hereditary factors because they breed true and produce uniform seed regardless of environmental conditions.

When certain environmental factors are necessary in addition to heredity, pigment formation is seen to be extremely complicated. Pigmentation may be brought about or it may not, a particular combination of factors other than heredity being necessary. Various environmental factors are important, but we find that some are more important than others. Emerson's (1) sun-red colors in maize are primarily dependent on light. Although these pigments will not form without sunlight, other environmental factors are also important.

Of the different factors that influence mottling in soy beans it is difficult at present to designate any one as the most important. A rich soil and a large supply of synthetic material for each seed is important, yet very small seeds may be badly mottled. On certain types of soil mottling has been entirely determined by the distance of spacing, but the rich soil in 1925 caused mottling even on closely spaced plants. Since the mottling pigments are formed toward the period of maturity an additional factor comes in. If maturation is brought about in a certain way the seed may be nonmottled, but if the plant continues to live after the seeds are mature the seed coats may be entirely black or brown.

SUMMARY

All varieties of the soy bean (*Soja max*) with yellow or green seed coats included in this study proved to be subject to mottling. A study of hereditary and environmental factors showed that both were important. Certain selections consistently produced less mottling than others, but badly mottled seed has been produced from selections where mottling was least expected by supplying favorable environmental conditions for the formation of pigment in the seed coat.

The black and brown pigments responsible for mottling were found to be glucosides, and an explanation of their production by means of an accumulation of sugars has been considered. The most striking evidence for this theory was obtained from the observation that mottling was greatly increased by reviving the growth of the plant after the seeds were practically mature.

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PIGEON PEA ANTHRACNOSE [1]

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INTRODUCTION

The pigeon pea, or gandul (*Cajan cajan* (L.) Millsp.) (*Cajanus indicus* Spreng.), is an important food crop in Porto Rico. The pods are picked before they mature, and the fresh, green seeds have a high nutritive value and may be found in the local markets almost throughout the year. The leaves and pods are eagerly eaten by all classes of livestock, and the crop is highly recommended as forage for cattle.

During the past three years pigeon peas at the station have been considerably damaged by a spotting of the pods, accompanied by molding and decay of the seeds. In general appearance the disease is strongly suggestive of bean anthracnose, and the term "anthracnose" was applied to it even before the causal organism was determined. Examination of the available literature failed to reveal any reference to a pod disease of pigeon peas, and the serious damage wrought by it seemed to warrant investigation to determine the cause.

The disease is rather generally distributed over the island, especially in the western section. Infected pods were received also from Fajardo, on the eastern coast, where the disease is said to have caused considerable loss.

SYMPTOMS OF THE DISEASE

Anthracnose of pigeon pea may be recognized by the appearance of spots or blotches on the leaflets, pods, and seeds. On mature leaflets the spots are dark brown, definite, somewhat angular, and usually delimited by veinlets. The spots are most conspicuous on the superior surface of the leaflet. With age the spots become reddish brown and often slightly zonate, sometimes coalescing and covering a large portion of the leaf surface. Single spots range in diameter from 2 to 7 millimeters. Infections occurring during the rainy season may cause spotting on any part of the leaf blade, whereas infections occurring during dry weather usually affect the midvein and extend into the leaf tissues on both sides. The spots are very similar to those caused by *Vellosiella cajani* (Henn.) Rangel (*Cerocspora cajani* Henn.), and a microscopic examination is often necessary to distinguish between them.

Infection of very young leaflets usually results in a blackening and shriveling of the veins, resembling lesions on *Phaseolus vulgaris* infected with *Colletotrichum lindemuthianum* (Sacc. and Magnus) B. and C. The leaves fall prematurely.

Circular to irregularly oval spots are formed on the pods. At first the spots are almost black, but later the center turns a dark brown and becomes surrounded by a narrow very dark purplish border.

[1] Received for publication Dec. 11, 1926; issued May, 1927.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 6
Mar. 15, 1927
Key No. Porto Rico-3

(589)

(Fig. 1.) When moisture is abundant the centers become salmon colored and mealy in appearance, owing to the formation of masses of conidia. The pod shows no cankering or pitting such as occurs with infection of garden beans by *Colletotrichum lindemuthianum*, probably because of the tough, chartaceous character of the pigeon pea. Infection of very young pods results in shriveling, twisting, and deformation, and in most cases the pods die and drop.

Injury of the seed varies often in the same pod from a shriveling caused by pod infection in the region of the placenta to complete·decay resulting from direct invasion by the fungus. In the latter case white tufts or a covering of white cottony mycelium usually may be seen. (Fig. 2.)
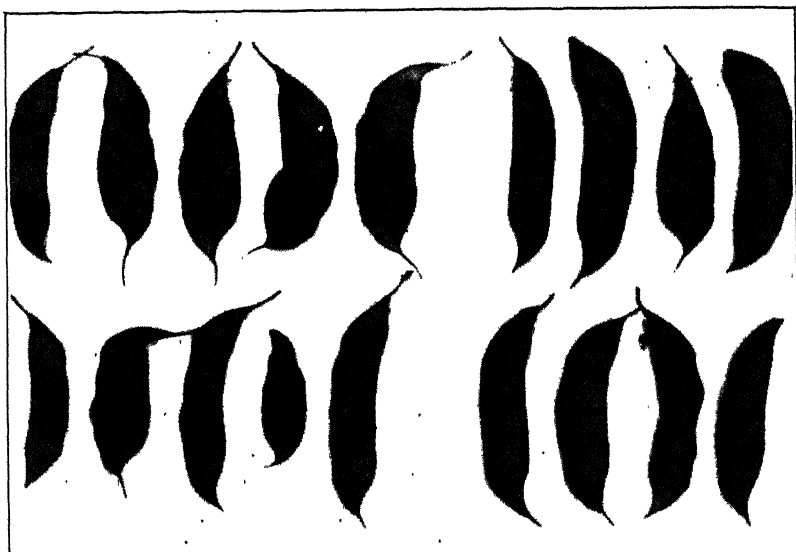
Fig. 1.—Left, pigeon-pea pods infected by anthracnose due to *Colletotrichum cajani*. The spotting and distortion of the pods is the most conspicuous symptom of the disease. Right, healthy pigeon pea pods

LOSSES CAUSED BY THE DISEASE

Damage attributable to anthracnose varies with the purpose for which the crop is to be harvested. When the pods are picked in the green stage the loss is much smaller than when they are allowed to ripen on the plant. Early in the dry season of January, 1925, 629 green pods were picked at random from plants in the station grounds. Of these 544, or 86.5 per cent, were found to be infected with anthracnose. The pods were mostly at the green-pea stage, at which time they are edible. The peas were shelled and graded as marketable or unmarketable; that is, discolored, moldy, decayed, or aborted. Table 1 shows the number of marketable seeds in diseased and healthy pods.

The 544 diseased pods yielded 1,342 marketable seeds; whereas, from the same number of healthy pods a yield of 2,118 seeds would be expected. The loss attributable to the fungus is 776 seeds, or 36.6 per cent.

In addition to the above easily calculated loss, there is the loss of young infected pods, which fall soon after the blossom drops. No exact data have been obtained on this kind of loss, but it is certainly considerable during the rainy season.
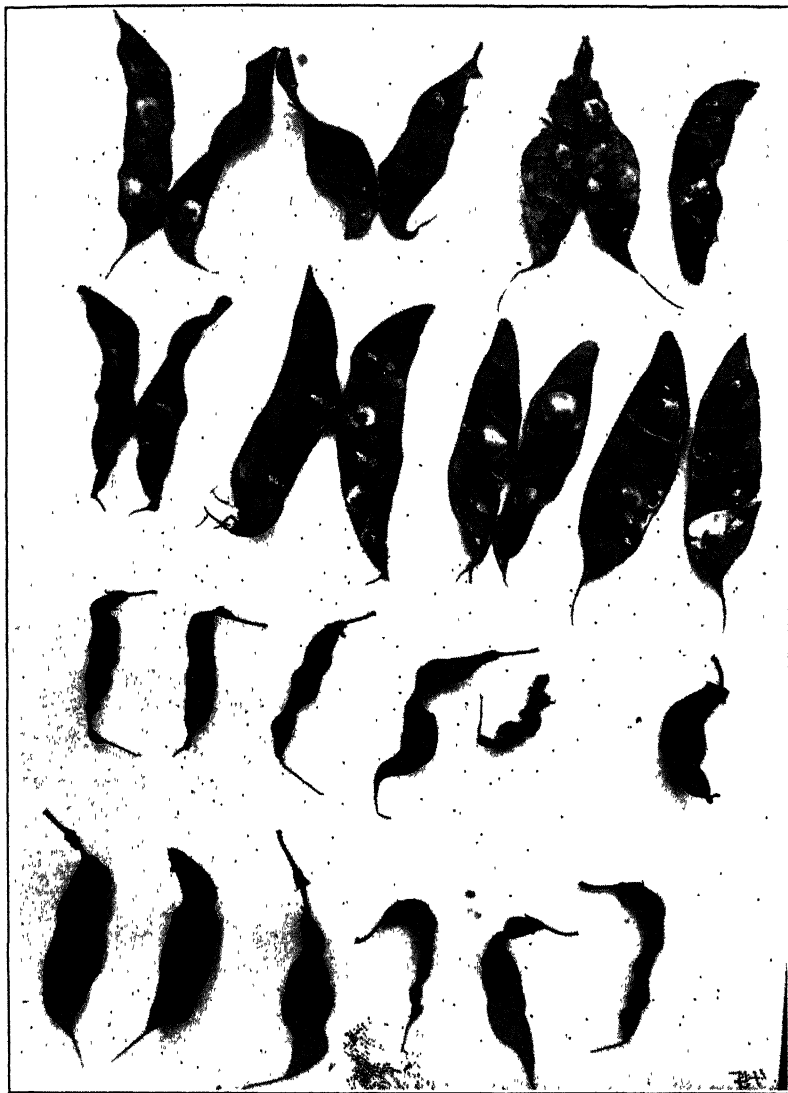
FIG. 2.—Above, split pigeon-pea pods bearing anthracnose spots. Note the white cottony mycelium. Some of the seed infection is confined to one or two dissepiments. Below, pods shriveled, distorted, and dead; the result of early infection by the anthracnose fungus

Probably the most important of the factors that determine the quantity of infection is rainfall. Pods which have formed during the rainy season show a much higher percentage of infection than those produced under dry conditions. Plants in sheltered situations produce a larger percentage of infected pods than plants to which the sunlight and breezes have free access.

TABLE 1.—*Marketable seeds in healthy and diseased pigeon-pea pods*

Marketable seeds per pod	Diseased pods in each class		Healthy pods in each class	
	Number	Per cent	Number	Per cent
0	66	12. 1	0	0
1	84	15. 4	0	0
2	101	18. 5	0	0
3	144	26. 5	21	24. 7
4	121	22. 3	52	61. 2
5	28	5. 2	12	14. 1
Total	544	100. 0	85	100. 0

Although the pods may become infected during any green stage, the resulting damage is greatly affected by the age of the pod at the time of infection. Early infections before the pod is 1 inch long usually cause the death of the whole pod. Later infections may cause the loss of the seed in the infected dissepiments only. The dissepiments of the pod are closed by the constrictions, and usually the fungus invades the seed in dissepiments on which acervuli are borne, but does not pass the constrictions. Late infections produce dotlike acervuli which do not enlarge much, and usually the fungus fails to penetrate to the interior.

THE ORGANISM

Microscopic examination of diseased pods and leaflets reveals the presence of a fungus of the anthracnose group. Acervuli are borne on the pod spots and on the superior surface of the leaf spots.

The conidia from pod acervuli are cylindrical, broadly elliptical to irregular in shape, usually rounded at the ends, hyaline when very young, but later becoming densely granular, or few—guttulate, straight, or slightly curved. They are continuous or very rarely 1-septate, and are 12 to 17 × 3.5 to 7.2 microns, with an average of 13.6 × 5.6 microns. (Fig. 3, B.)

The conidia are borne in abundance on pod acervuli and in smaller numbers on the leaf acervuli.

The conidiophores are simple, hyaline, cylindrical or slightly swollen, usually septate near the base, rounded at the apex, and 15 to 25 × 3 to 5.5 microns. (Fig. 3, A.)

Setae are numerous on old pod spots, but occur only occasionally on the leaves. They are fasciculate, dark brown, cylindrical, usually somewhat curved or bent, rounded at the apex, 1 to 5 septate, and 70 to 120 × 3.3 to 4.2 microns, with an average of 100 × 3.5 microns. (Fig. 3, D.)

The above description of the fungus places it in the genus Colletotrichum. Two references to the occurrence of this genus on the pigeon pea have been found. Dash (*4, p. 38*) stated,[2] "A species of Colletotrichum was found on pigeon pea at Codrington [Barbados] which seemed to cause death to the branches attacked." Rangel (*6, p. 154–155*) in Brazil described and figured a Colletotrichum on leaf spots of the pigeon pea. His description follows:

[2] Reference is made by number (italic) to "Literature cited," p. 596.
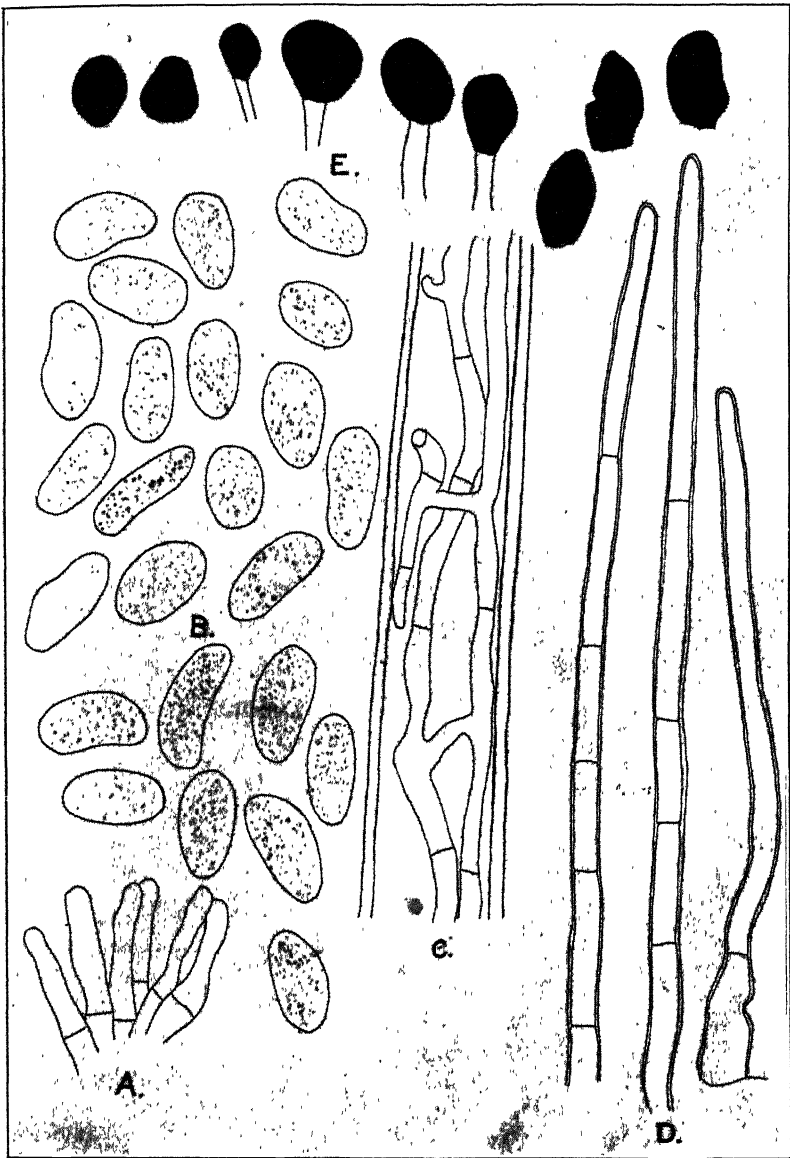
FIG. 3.—*Colletotrichum cajani*: A, conidiophores; B, conidia; C, section of a hair from an infected pigeon-pea pod, showing invasion by the mycelium of the fungus; D, setae from a pod acervulus; and E, appressoria from 2-months-old potato-dextrose agar culture. × 1175

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Colletotrichum cajani, Rangel (n. sp.).
' Maculis majusculis, apicalibus, obscure ferrugineis, indeterminatis vel atro-brunneo marginatis; acervulis plerumque epiphyllis, minutis, olivaceis; conidiis cylindraceis, oblongis vel lato ellipsoideis, continuis, granulosis, hyalinis, 10—15=4—5μ setulis rectis vel curvulineis, septatis, brunneolis, 45—60=4—5μ. In foliis vivis *Cajani indici*. Niteroy. Brasiliae. (Exs. 723. Maio. 1913).

The fungus here described does not differ essentially from that under discussion. As to the differences in the size of conidia and setae, it should be remembered that Rangel's observations were made on material from leaf spots, whereas the writer used diseased pods on which the conidia and setae developed more profusely. The fungus under discussion is therefore referred to *Colletotrichum cajani* Rangel.

In culture the fungus grows rapidly on a variety of nutrient media. On potato-agar plates the growth is closely appressed, thin, white, spreading, regular, radiate and sliming from below. At 25° C. the mycelia after 72 hours average 22 millimeters in diameter, and after 120 hours, 37 millimeters. No spores were produced during one week.

On potato-dextrose agar plates the growth has the same characters as on potato agar except for a slight increase in density and in rapidity of growth. After 72 hours the mycelia average 25 millimeters in diameter, and after 120 hours, 46 millimeters. After one week no conidia were borne.

On oatmeal-agar plates the growth was intermediate in density and rapidity between that occurring on potato agar and potato-dextrose agar. After 72 hours the mycelia average 23 millimeters in diameter and after 120 hours, 43 millimeters. No conidia were borne in one week.

On steamed pigeon-pea pods a luxuriant growth of white cottony mycelium is made. Conidia are abundant after a week's growth. The conidia are very similar in size and shape to those borne on pods as the result of natural infection.

In 2-months-old cultures on potato-dextrose agar and pigeon-pea pods, appressoria, as described and figured by Edgerton (5) are abundant. They are dark brown in color and are formed by the swelling and thickening of the wall of an apical hyphal cell. They usually occur singly, but occasionally two to five apical cells become swollen, producing the appearance of a chain of chlamydospores. The appressoria are oval to irregular in shape and vary in size, ranging from 6.4 to 12 × 4.8 to 6.4 microns.

Edgerton (5) found appressoria occurring generally in the anthracnose fungi following the germination of conidia in a medium lacking nutrient material. In the writer's potato-dextrose agar cultures conidia were never numerous, yet appressoria appeared in large numbers, and observations indicate that at least a large majority were formed directly from hyphal cells. The resulting thick-walled cells or resting bodies seem in every respect to be analogous to chlamydospores.

INOCULATIONS

The fungus was grown on steamed pigeon-pea pods for two weeks, with profuse conidia production. A water suspension of conidia and mycelium was atomized on 107 pigeon-pea seedlings about 8 inches high. The seedlings were then watered daily by sprinkling and kept shaded for 48 hours. The earliest symptoms of infection appeared about five days later, when the veins in some of

the youngest leaves darkened. After 12 days infection was well advanced. Apparently the leaflets emerging from the bud during inoculation were most easily infected. Of 107 inoculated seedlings, 102 showed unmistakable symptoms of infection. In every case infection appeared on the veins of the leaflets, resulting in death in severely affected specimens. Deformed leaflets were borne by other seedlings as a result of the continued growth of the healthy portions and the arrested growth of infected veins. Infection of the older leaflets was not common. When infection did occur, the blades were spotted. The older leaflets showed no vein infection. Elongated narrow stem lesions were present on 54 seedlings. The lesions were as yet hardly more than a darkening of the epidermal cells. The woody character of the pigeon-pea stem prohibits the formation of cankers such as occur on anthracnose-infected garden beans. Petiole infections occured on 25 seedlings and usually caused the affected leaflets to yellow and fall.

The fungus was reisolated from vein, stem, and petiole lesions and was found to be identical morphologically and in cultures with the inoculating organism.

Thirty-eight seedlings were used as controls. These received the same treatment as was given the inoculated plants except that sterile water was substituted for the inoculating fungous suspension. Thirty-four seedlings remained healthy, two were doubtful, and two became slightly infected.

Garden-bean seedlings were inoculated like the pigeon-pea seedlings, the pigeon-pea seedlings being used for parallel inoculations as a check on the virulence of the inoculating cultures. The varieties of *Phaseolus vulgaris* used included Giant Stringless Green Pod, Valentine Pencil-Pod Black Wax, and Refugee (all bush beans). These varieties were selected because of their susceptibility to *Colletotrichum lindemuthianum*. Barrus (1) showed them to be susceptible to the alpha and beta strains, and Burkholder (3) demonstrated their susceptibility to the gamma strain.

No infection was obtained on the varieties named, although the parallel inoculations on pigeon peas produced abundant leaflet-vein infection.

The infection of varieties of *Phaseolus vulgaris* with a Colletotrichum from *Cajan cajan* could hardly be expected in view of the results obtained by Barrus (2). Summarizing the results of extensive inoculation experiments, he states: "There are evidently but few species outside the genus Phaseolus which are susceptible in any degree to anthracnose [*C. lindemuthianum*], and no plants except varieties of *Phaseolus vulgaris* are susceptible to such an extent that the disease becomes epiphytotic in regions where such plants are extensively grown."

SUMMARY

The pigeon pea (*Cajan cajan*) in Porto Rico is attacked by anthracnose, resulting in spotting of the pods and leaves and destruction of the seeds.

On young leaves infection occurs mostly on the veins and causes them to blacken and shrivel; on young pods infection causes distortion, abortion, and death.

The losses sustained are due to the destruction of young pods and the decay or discoloration of one to all the seeds in infected pods. Infection is most serious during periods of heavy rainfall.

The causal organism is referred to *Colletotrichum cajani* Rangel, which is now first recorded as the cause of a pod and seed disease.

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PRELIMINARY STUDIES ON THE RELATION OF FIRE INJURY TO BARK-BEETLE ATTACK IN WESTERN YELLOW PINE[1]

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INTRODUCTION

That the effect of fire in pine forests of the Pacific slope region has been studied in many of its aspects is indicated by the published information on this problem. One phase, which has been only briefly discussed in the literature now available, is that of bark-beetle infestations which follow in the wake of fires.[2]

It has been generally recognized that there is a direct relation between fire injury and subsequent insect damage on burned-over areas. Two types of loss are involved—destruction of the merchantable value of fire-killed trees by wood-boring insects, and the actual killing by bark beetles of trees that survived the fire.

An increase in the number of trees infested by bark beetles is a frequent aftermath of the so-called "light" fires which rarely kill mature trees. A very perceptible flare-up of the infestation in a burned-over area frequently follows a fire of this character. Many trees only moderately injured by fire and apparently capable of recovering will succumb to the attacks of these beetles.

Such damage follows as a result of a sporadic local increase of bark-beetle population within the fire area which can be explained only by the assumption that numbers of beetles fly into the area from the surrounding forest. Because of fire injury certain trees become especially attractive to the beetles. The physiological basis for this attraction is but vaguely understood. The odor of fire-scorched foliage and cambium may be an attractive influence, or the insects themselves may possess an instinctive ability to select those trees in which sap resistance has been weakend by fire injury, but whatever the influence, it is evidently a very strong one in determining the behavior of Dendroctonus beetles.

The multifold aspects of the interrelation of fire and insect damage raise many questions that are pertinent to the protection of pine forests. What type and what degree of fire injury make trees attractive to bark beetles? Are such trees capable of recovery if not attacked by insects? Do bark beetles "breed up" in fire areas, increasing their numbers to an epidemic status, and then become aggressive in uninjured trees in and around burned areas? The answers to these questions can be found only by a careful study and analysis of the infestations which develop within and adjacent to

such areas. Since 1917 the Bureau of Entomology has had studies of this character under way in the yellow-pine regions of California and Oregon.

The results now available indicate the tendencies to be expected under the conditions where these fires occurred. Rarely do any two fires occur under identical conditions, and each presents its own individual complex of factors which may modify results. It can not be claimed therefore that the results which are given in this paper will be found applicable to all fires under any conditions; they are intended only to serve as a contribution toward the information which is being accumulated on this problem.

THE MISTLETOE BURN

GENERAL CONDITIONS OF THE AREA

One area in which the more intensive studies have been carried on is known as the Mistletoe burn in southern Oregon. This fire, which occurred October 31, 1917, burned over 800 acres on the slopes of the Siskiyou Mountains, 7 miles southeast of the town of Ashland. The elevations within the burn range from 3,000 to 4,000 feet above sea level, with exposures of the slopes mainly to the south and east. The timber type consists of a mixed stand of yellow pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga taxifolia*), with a small amount of sugar pine (*Pinus lambertiana*) intermixed. At the lower elevations and on the poorer sites brush cover predominated, which graded into fairly well-stocked mature stands of conifers on the more favorable slopes. There was abundant reproduction and new growth of conifers on the better sites.

From a 100 per cent cruise of the burned-over area, the volume of merchantable timber of the more important species was found to be as follows: Yellow pine, 44.5 per cent; Douglas fir, 52.5 per cent; sugar pine, 3 per cent.

STUDIES OF AREA PRECEDING FIRE

Both the area that was burned and the surrounding forest had been under observation since 1914. In a study begun in 1914 to determine the progress of the bark-beetle infestation in the general region, annual cruises had been made in which all insect-killed yellow pine and sugar pine were marked and records taken from each tree. Although the fire started unexpectedly from unknown origin, the status of the bark-beetle infestation at the time of the fire was definitely known.

CHARACTER OF THE FIRE

In the open and mature stands the damage was typical of a ground fire of moderate severity. Seedlings and small reproduction were killed, a few butt scars were newly formed on the larger trees, and old scars were enlarged, but no yellow pine trees of merchantable size were killed outright.

On the steeper slopes, however, the fire developed considerable intensity in the thickets of new growth and in brush cover and either killed or consumed all the forest cover. In these sections of the area, where brush and trees of pole sizes were intermixed, the fire reached the crowns, burned away the foliage, and killed outright a considerable part of the stand.

CRUISE OF FIRE DAMAGE

The following year (1918) a cruise was made of the entire burn to determine the amount of damage to merchantable trees. As a basis for a study of subsequent insect attacks, all trees on the area were marked according to the nature of fire injury. A base line was run across the burn and the entire area was covered by strips, one tally wide, run at right angles to this base line. All trees over 10 inches d. b. h. were examined and classified according to the degree of fire injury and were marked for future reference according to class of injury. This was done by smoothing off the outer bark with an ax and writing the number of the class with a black crayon. At the same time cruises were made in the surrounding areas and the loss was analyzed by zones from 1 to 6 miles distant from the center of the burn. A similar cruise was made in November, 1919, and a final one in 1920 (fig. 1).

LIMITATIONS OF DATA

This study was planned to determine the relation of the fire-injured trees to subsequent bark-beetle attack and it was found unnecessary to consider trees under 10 inches d. b. h. No quantitative data were taken on the destruction of seedlings, reproduction, brush, and other cover not of a merchantable character, although this loss was a factor in the total amount of damage. Douglas fir also was thrown out of consideration when it was found that the only insects which attacked it were secondary borers and that their attacks were confined largely to fire-killed trees. The percentage of sugar pine was so small as to be of practically no importance. The study, therefore, is confined entirely to the infestation of the western pine beetle (*Dendroctonus brevicomis* Lec.), which developed in the fire-injured western yellow pine. In discussing the losses caused by this beetle, only those trees killed by its attacks are considered.

TABLE 1.—*Yellow pine on Mistletoe burn classified according to fire injury*

Class	Number of trees	Per cent	Volume (board feet)	Per cent
Class 1, uninjured by fire	1,577	65.7	1,385,430	64.7
Class 2, light fire injury	573	23.9	539,370	25.2
Class 3, medium fire injury	113	4.7	120,360	5.6
Class 4, heavy fire injury	57	2.4	80,450	3.7
Class 5, killed by fire	80	3.3	16,720	.8
Total	2,400	100.0	2,142,330	100.0

Trees in classes 2, 3, and 4 are those which survived the fire but with different degrees of injury (Table 1). In all three classes the bole of the tree was more or less blackened, and in classes 3 and 4 the cambium also was damaged. In class 2 the foliage was partly scorched, causing the needles to discolor. In class 3 practically all of the crown was scorched, but not sufficiently to kill the terminal buds on lateral limbs and top. In class 4 the entire foliage was burned away and the terminal buds killed, but the cambium still remained green at the time of examination six months after the fire. In class 5 an examination near the base showed that the cambium had been deadened by the first effects of the fire, so that these trees were unquestionably fire killed. Figure 2 illustrates the comparative degrees of fire injury used as a basis for this classification. Photographs of trees in classes 3 and 4 are shown in Figure 3.

FIG. 1.—Distribution of trees killed by bark beetles (*Dendroctonus brevicomis*) in the Mistletoe burn and in the surrounding zones one year before and three years after the fire
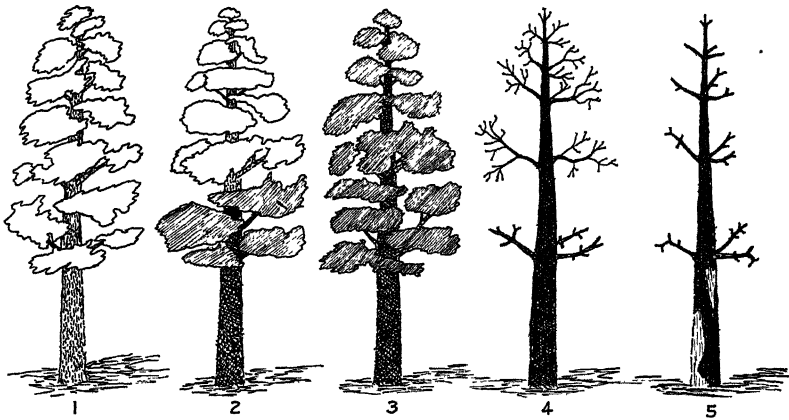
FIG. 2.—Five trees, each illustrating one of the five classes represented on the Mistletoe burn: 1, Uninjured by fire; 2, light fire injury; 3, medium fire injury; 4, heavy fire injury; 5, killed by fire
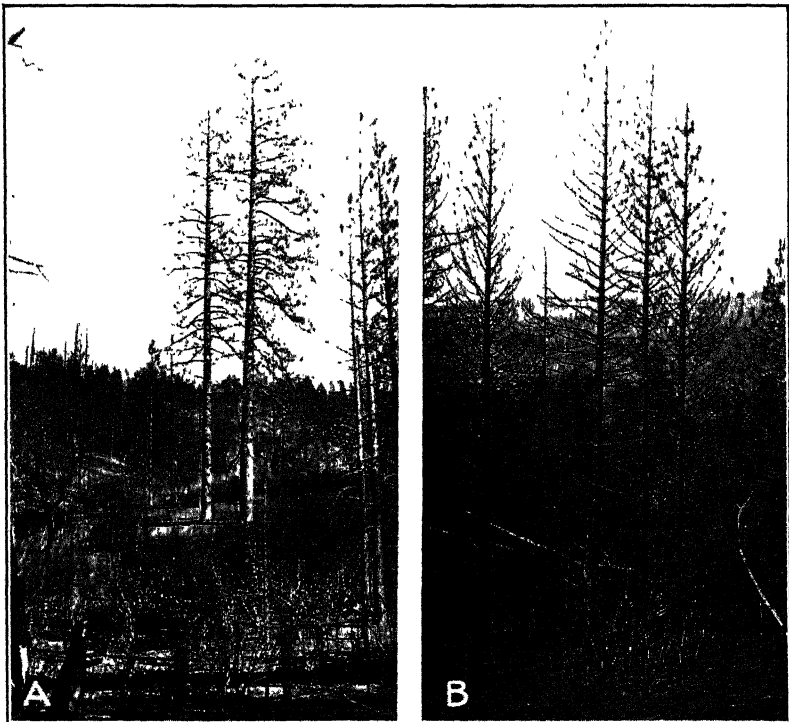
FIG. 3.—Fire-injured trees which attract bark-beetle attacks. A, class 3. Two fire-injured trees, showing base and middle portions of trunks fire charred and almost all of foliage scorched. The buds of the new needle growth were uninjured toward the top of the tree. The larger trees suffering this degree of fire injury have some chance to recover if not attacked by insects. They are, however, more attractive to bark beetles than trees in any other class. Over 80 per cent of the trees in this class were attacked the first season after the fire. B, class 4. A group of three small trees showing heavy fire damage. The trunks of these trees were scorched for their entire length, and the greater part of the foliage was burned away. The terminal buds were killed, although the cambium remained green until the following season. This class is attractive to bark beetles only during the first season after the fire. Trees injured to this extent eventually die as a result of the fire injury, even though they escape bark-beetle attack

Butt scars were considered, but as few of these were formed by this fire and no trees so injured were attacked by bark beetles, this class of damage has been left out of consideration in order to simplify the analysis of other factors.

Trees entirely killed by the fire average considerably smaller than those composing the other classes, as is shown by the fact that while 3.3 per cent of the number of trees were killed by the fire, these amounted to only 0.7 per cent of the volume.

VOLUME OF TIMBER KILLED BY BARK BEETLES WITHIN THE BURN

From May 1 to October 31, 1917, the season preceding the fire, the western pine beetle killed 20 yellow pine trees within the area of the burn, containing a volume of 12,500 board feet (Table 2). No attacks occurred in November, 1917, following the fire. This is explained by the fact that at that time the attacks of the overwintering broods had already occurred and beetles would not fly again until the following spring.

During 1918 attacks occurred in many trees within the burn. Wherever fire-injured yellow pine predominated, heavy centers of infestation formed. The volume killed within the burn amounted to 200 board feet per acre, whereas in the surrounding area for a distance of 6 miles the loss averaged 29 board feet per acre. During 1918 a volume of 159,720 board feet was destroyed within the burn— an increase over 1917 of 1,000 per cent.

In 1919 the loss was reduced to 42,180 board feet. This represents a reduction of about 74 per cent. In 1920 the loss was further reduced to 15,680 board feet.

The course of the infestation within the burn was marked by a very pronounced increase during the season following the fire and a subsidence within the two following seasons to a point about as low as that which existed before the fire.

VOLUME OF TIMBER KILLED BY BARK BEETLES ON AREA SURROUNDING BURN

Outside of the burned area the volume of infested timber followed a course during the seasons of 1918, 1919, and 1920 quite different from that within the burn. The yellow pine type of this locality consists of a strip extending along the slopes of the Siskiyou Mountains between the elevations of 2,000 and 5,000 feet. Open valleys predominate below these elevations, while above 5,000 feet the forest type is composed almost entirely of fir and subalpine species. As a result of this type distribution, the area susceptible to pine-beetle infestation consists of a narrow belt from 2 to 3 miles in width extending from northeast to southwest.

The relation of the surrounding zones to the fire area is shown in Figure 1. The fire area formed an irregular L-shaped strip approximately 3½ miles long by one-half mile wide. The axis of this area was assumed to be the center of the burn. From this point the first zone was defined as a circle with a 1-mile radius. The second zone was drawn from the same center with a 2-mile radius, and included the area within the outer limit of this circle and the outer boundary of the first zone. The third, fourth, fifth, and sixth zones were laid out concentrically in the same manner.

TABLE 2.—*Progress of bark-beetle infestation on fire area and on surrounding area*

Year	Fire area, 800 acres		Surrounding area, 18,500 acres	
	Number of trees	Volume (board feet)	Number of trees	Volume (board feet)
1917	20	12,500	449	403,670
1918	220	159,720	508	516,130
1919	50	42,180	224	199,210
1920	17	15,680	181	160,980
Total	307	230,080	1,362	1,279,990

The yellow pine type, however, extended in the form of a long narrow strip about 2½ miles in width along the flanks of the Siskiyou Mountains, so that only part of the area in each zone was covered with timber susceptible to attack by the western pine beetle. The area of pine type in each zone, therefore, was more or less irregular and does not conform to the total areas of the concentric circles defining the limits of the zones. However, it is the distance to which the effects of the fire influenced the infestation in the surrounding pine area that is the important factor under consideration, and this is definitely determined by the boundaries of the various zones established.

All of the area included within these zones had been cruised before the fire, the insect-killed trees had been marked and located by map, and individual tree records taken. This same system of marking was continued for three years after the fire. The data thus collected were compiled according to the zone in which the loss occurred.

The records thus obtained show that for the general area surrounding the burn there was a slight tendency toward increase of infestation during the season of 1918, but at the same time the zones within 3 miles of the burn registered a perceptible decrease. During 1919, however, when the volume of infestation within the burn decreased 74 per cent, the first zone immediately adjoining showed an increase of nearly 60 per cent, while in all zones beyond this there was a very marked decrease during the season (Tables 3 and 4).

TABLE 3.—*Number of trees killed and loss in board feet in fire area and in each surrounding zone from 1917 to 1920, inclusive*

Fire area and surrounding zones	Number of trees killed and volume destroyed							
	1917		1918		1919		1920	
	Trees	Volume (board feet)	Trees	Volume (board feet)	Trees	Volume (board feet)	Trees	Volume (board feet)
Fire area	20	12,500	220	159,720	50	42,180	17	15,680
Zone 1	47	42,255	31	31,500	56	49,800	30	26,680
Zone 2	122	109,680	104	105,665	44	39,120	38	33,820
Zone 3	82	73,720	74	75,180	47	41,800	42	37,350
Zone 4	68	61,135	90	91,440	41	36,450	38	33,780
Zone 5	71	63,830	129	131,065	22	19,580	30	26,680
Zone 6	59	53,050	80	81,280	14	12,460	3	2,670
Total	469	416,170	728	675,850	274	241,390	198	176,660

TABLE 4.—*Annual loss per acre on the fire area and on each of the surrounding zones for one year previous to fire and for three years after*

Fire area and surrounding zones	Acreage of yellow pine type in each zone	Board-foot loss per acre			
		1917	1918	1919	1920
Fire area	800	16	200	53	20
Zone 1	920	46	34	54	29
Zone 2	3,440	32	31	11	10
Zone 3	4,960	15	15	8	8
Zone 4	4,000	15	23	9	8
Zone 5	3,240	20	40	6	8
Zone 6	1,200	44	68	10	2

From this evidence it would appear that the natural tendency of the infestation was to increase during 1918 and to decrease during 1919. These natural tendencies were disturbed, however, by the attractiveness to the beetles of the fire-injured trees in 1918, which caused many beetles to leave the first three surrounding zones and concentrate in the burn. In 1919, as the attraction of the fire-injured trees had subsided, some of the beetle population which had concentrated in the burn attacked trees in the area immediately adjoining, causing a perceptible increase locally although all other areas registered a decrease. A comparison of the course of the infestation both inside and outside of the burn is shown in Figure 4 and Table 4.

CLASSES OF FIRE-INJURED TREES ATTACKED

The selection made by the beetles in the various classes of fire-injured trees is shown in Figure 5, A. There was a decided tendency on the part of the beetles to select trees in classes 2 and 3 which sustained light to medium fire injury. Some attacks occurred in trees of class 1 which were uninjured in any way by the fire, but it is evident that trees which sustained a light to medium fire injury were far more attractive to the beetles than any other trees on the burned area. These were trees which appeared to be likely to recover from the effects of the fire itself.

FIG. 4.—Comparative rates of loss, in board feet per acre, of timber killed by bark beetles on burned area and on each of the surrounding zones during the season preceding the fire and for three seasons subsequent to it (Mistletoe burn)

TABLE 5.—*Number of trees of each class attacked and loss in board feet during 1918, 1919, and 1920*

Class	1918 (first year after fire)		1919 (second year after fire)		1920 (third year after fire)	
	Trees	Volume (board feet)	Trees	Volume (board feet)	Trees	Volume (board feet)
1 (uninjured by fire)	17	24, 370	21	16, 050	12	8, 560
2 (light fire injury)	81	60, 140	11	8, 260	3	2, 620
3 (medium injury)	84	49, 910	18	17, 170	2	4, 500
4 (heavy injury)	38	25, 300	0	0	0	0
5 (fire-killed)	0	0	0	0	0	0
Total	220	159, 720	50	41, 480	17	15, 680
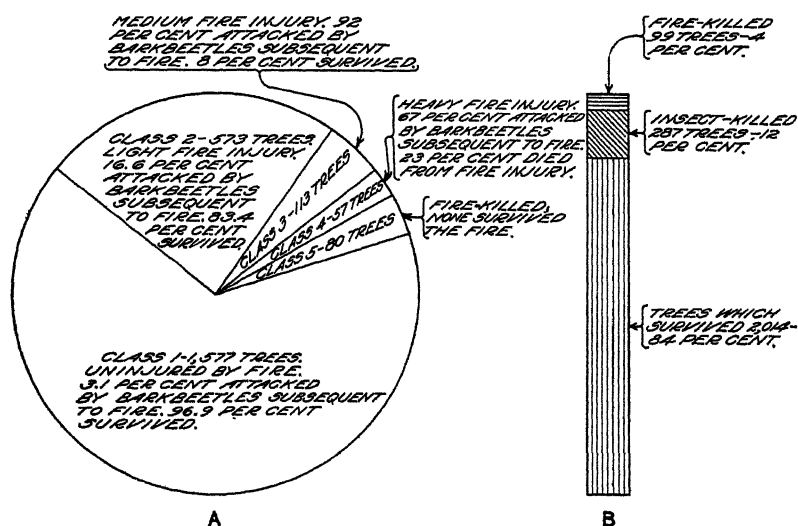
FIG. 5.—Relative amounts of fire and insect damage following the Mistletoe fire. A, relative number of trees in each class of fire injury and percentage of each class attacked by bark beetles; B, percentages of surviving, insect-killed, and fire-killed trees

TABLE 6.—*Number of trees of each class attacked and percentage of annual infestation in each class, during 1918, 1919, and 1920*

Class	1918		1919		1920		Total for period	
	Number of trees	Percentage of infestation	Number of trees	Percentage of infestation	Number of trees	Percentage of infestation	Number of trees	Percentage of infestation
1	17	8	21	42	12	71	50	17.5
2	81	37	11	22	3	17	95	33
3	84	38	18	36	2	12	104	36
4	38	17	0	0	0	0	38	13
5	0	0	0	0	0	0	0	0
Total each year	220	100	50	100	17	100	287	100

PERIOD OF ATTRACTION TO TREES WITHIN THE BURN

Trees in classes 1, 2, and 3 were attacked during each of the three years following the fire. (Tables 5 and 6 and Fig. 6.) In classes 2 and 3 by far the greater part of these attacks, or more than 80 per cent, occurred during the first year after the fire. During the second and third years the attacks in trees of these classes were still relatively greater than the attacks in uninjured trees but to a much less extent than during the first season.

All attacks in class 4 occurred during the first season following the fire. The fact that no attacks occurred during the second and third seasons after the fire is readily explained, as all trees in this class that were not attacked by beetles during the first season died as a result of fire injury before the period of attack during the second season.

While the period of attraction to fire-injured trees was extended during the second and third seasons, the main concentration of beetles within the burn occurred during the first season after the fire.

FIG. 6.—Relative number of trees attacked annually on Mistletoe burn by bark beetles in each class of fire injury for three seasons subsequent to fire

DEVELOPMENT OF BROODS IN FIRE-INJURED TREES

EFFECT OF FIRE ON BROODS IN OUTER BARK OF INFESTED TREES

There were six yellow pine trees infested with broods of the western pine beetle standing within the burn at the time that the fire occurred. The broods in these trees consisted mainly of full-grown larvae and a few pupae in the outer bark. All of these trees were exposed to heat from a ground fire of moderate intensity, and the outer bark near the base received more or less scorching. In five of these trees the broods continued to develop after the fire and a normal emergence occurred from them the following spring. In one tree, however, the broods in larval and pupal stages were killed on one side of the trunk near the base where the heat had been severe.

MORTALITY OF BROODS IN TREES ATTACKED AFTER FIRE

During the season of 1918 it was found that many broods of the western pine beetle in the fire-scorched trees were dying in the early

larval stages. In trees where this high mortality occurred it was noted that an extremely moist condition of the inner bark was prevalent. The cause of this excess of moisture has not been definitely determined. Apparently it resulted from the sudden cutting off of the respiratory functions of the tree due to the defoliation caused by fire. Much of the moisture in the sapwood and cambium condensed in the inner bark, resulting in conditions highly unfavorable to the young larvae of the western pine beetle. The term used by cruisers to designate this condition is "sour sap."

In order to obtain some idea of the extent of this mortality, a study was made of the number of beetles attacking the bark surface of these sour-sap trees as compared with the subsequent progeny which emerged. A total of 272 square feet of bark was collected from 28 trees which had been attacked and abandoned by the beetles, or an average of nearly 10 square feet to the tree. An effort was made to select fairly representative trees, and the bark sections were collected at different heights from each tree ranging from 6 to 70 feet from the base.

The holes through which pairs of beetles entered the cambium were counted, as were also the exit holes from the same bark sections. Previous studies had determined that the number of beetles going in through each entrance hole averaged 2.59. Upon this basis it was possible to arrive at the following comparison of the number of beetles that entered and the number that emerged from fire-injured trees.

Number of square feet of bark analyzed_____ 272
Total number of entrance holes_____ 1, 372
Total number of beetles attacking (1,372×2.59)_____ 3, 553
Total number of progeny emerging (exit holes)_____ _____ 3, 277
Reduction in emergence over attack_____ 276

This analysis indicates that although the fire-injured trees attracted beetles to such an extent as to cause a concentration within the burn, at the same time they afforded a very unfavorable breeding ground for the insects and in the end contributed toward an actual reduction of their numbers.

DEVELOPMENT OF BROODS IN TREES ATTACKED OUTSIDE OF BURN

A similar study was made of bark from trees outside of the burn in order to compare the mortality of the beetles in fire-injured trees with that of beetles in normally infested trees. A total of 341 square feet of bark was collected from 76 trees. The results of these counts were as follows:

Number of square feet of bark analyzed_____ _____ 341
Total number of entrance holes_____ 2, 048
Total number of beetles attacking (2,048×2.59)_____ 5, 294
Total number of progeny emerging (exit holes)_____ 22, 807
Excess of emergence over attack_____ 17, 513

A direct comparison of the average attacks and emergence per square foot unit on fire-injured and normal trees is as follows:

On fire-injured trees: Five entrance holes; 13 beetles attacking, 12 emerging; that is, a decrease of 1 beetle, or 7.7 per cent.

On normal trees: Six entrance holes; 16 beetles attacking, 67 emerging; that is, an increase of 51 beetles, or 318.8 per cent.

A graphic comparison is also shown in Figure 7. In normal trees there was an increase during 1918 of approximately 320 per cent, whereas in the fire-injured trees there was an actual decrease of about 8 per cent. To this extent the fire acted as a controlling factor by reducing the number of beetles that were attracted into the burned area.

SUMMARY OF RESULTS ON MISTLETOE BURN

FIRE DAMAGE

Of the total merchantable pine volume on the area covered by this burn, 0.7 per cent was immediately killed by the fire; 3.7 per cent was scorched to the point of complete defoliation, so that recovery was improbable; 30.6 per cent had the foliage partially scorched, but apparently was capable of recovery; the remaining 65 per cent was not visibly injured by the fire.

INSECT DAMAGE

This fire, which occurred late in October, produced no disturbance of the bark-beetle infestation in and around the area during the remainder of that season.

Throughout the season following the burn, fire-injured trees formed an attraction which caused a concentration of beetles in the burned-over area. As a result, the volume of infested timber within the burn increased 1,000 per cent over that on the same area during the preceding year.

The attraction of the beetles to individual trees was largely determined by the character of the fire injury. A decided preference was shown for trees in which the lower trunk and foliage had been moderately or lightly scorched. This attraction was most pronounced during the first year after the fire.

FIG. 7.—Comparison of brood development in trees under normal conditions adjacent to Mistletoe burn and in fire-injured trees

INFLUENCE OF FIRE UPON SUBSEQUENT INFESTATION

This concentration of beetles within the burn did not cause an epidemic infestation that continued to increase within the burn or to spread to surrounding areas. During the second year after the fire the infestation both in the burn and in the surrounding area returned to a status approaching that which existed before the fire.

The volume of infestation in the surrounding area was apparently influenced by developments within the burn. During the year following the fire the volume of infestation in the zones immediately adjoining and within 3 miles of the burn was reduced, apparently because of the attraction of beetles to the fire-injured trees in the burned-over area. The infestation in zones more than 3 miles distant from the burn increased slightly during the same season.

A study of bark collected from beetle-attacked fire-injured trees showed that on account of excessive moisture in the inner bark a heavy mortality occurred in the western pine beetle broods. A comparison indicates that the number of beetles emerging from fire-injured trees was 8 per cent less than the number of parent adults which attacked, resulting in a slight reduction of the second generation of the beetle population in the burned area.

THE SISKIYOU BURN

The Siskiyou burn was studied in the same detail as the Mistletoe burn just described. The fire occurred on August 15, 1918, and covered about 200 acres situated approximately 4 miles south of the Mistletoe unit.

Type and slope conditions were quite similar to those on the Mistletoe burn, but the fire was of somewhat greater severity as shown by the correspondingly greater damage to mature trees. Cruises were made in which the fire-injured trees were classified on the same basis as in the Mistletoe burn (Table 7).

TABLE 7.—*Yellow pine on Siskiyou burn classified according to fire injury*

Class	Number of trees	Per cent
1 (uninjured by fire)	176	31
2 (light fire injury)	205	37
3 (medium fire injury)	52	9
4 (heavy fire injury)	114	20
5 (killed by fire)	18	3
Total	565	100

At the time of the fire there were two yellow pine trees with a volume of 260 feet which had been infested by the western pine beetle during the early summer preceding the fire. These trees represented the first seasonal generation and were nearly abandoned at the time. Within a few days after the fire there was a concentration of beetles on the injured trees in the burn. In this respect the effects of the Siskiyou fire differ from those of the Mistletoe area, where the beetles did not concentrate until the season following the fire. This difference is easily explained by the fact that the Siskiyou fire occurred during the seasonal flight period of the beetles while the Mistletoe fire occurred in the fall after this activity had subsided.

During the latter part of August and through September the beetles killed 41 new trees having a volume of 44,130 board feet, an increase of 1,950 per cent over the number killed by the preceding generation (Table 8).

TABLE 8.—*Loss from infestation of the western pine beetle in the Siskiyou burn*

Season of attack	Number of trees	Volume (board feet)	Increase or decrease in number of trees attacked	
			Increase	Decrease
			Per cent	*Per cent*
1918, first generation before fire	2	260		
1918, second generation after fire	41	44,130	1,950	
1919	62	74,990	51	
1920	4	3,150		93.5

During 1919 the insects continued to concentrate in the fire area and the loss continued, increasing 51 per cent over that of 1918. In 1920, however, the third year after the fire, there was a pronounced decline in attack and the loss for that season averaged 93.5 per cent less than that for 1919.

Table 8 shows that the infestation on this burn increased after the fire in about the same degree as that on the Mistletoe burn and that the cycle of increase and decrease was of practically the same duration. The heavy increase immediately after the fire was followed by an equally pronounced decrease within two seasons.

This infestation within the burn was distributed in the different classes of trees as shown in Table 9.

TABLE 9.—*Distribution of infestation in different classes of trees in the Siskiyou burn*

Class	1918			1919			1920		
	Number of trees	Volume (board feet)	Per cent of total volume	Number of trees	Volume (board feet)	Per cent of total volume	Number of trees	Volume (board feet)	Per cent of total volume
1	2	1,110	2.5	2	2,370	3	0	0	
2	8	8,680	19.7	15	13,050	17	0	0	
3	6	11,260	25.5	6	2,250	3	4	3,150	100
4	19	4,790	10.9	39	57,320	77	0	0	
5	6	18,290	41.4	0	0	0	0	0	
Total	41	44,130	100.0	62	74,990	100	4	3,150	100

COURSE OF INFESTATION IN SURROUNDING AREAS

The Siskiyou fire was so located that a study of the surrounding infestation by zones was not practicable. However, the general course of the infestation in the surrounding region can be determined by the figures showing the same progress in the area surrounding the Mistletoe burn.

No study was made of brood mortality in trees either within or without this fire area, but as the effects of the fire differ but little from those on the Mistletoe burn, there is reason to believe that the same high mortality in fire-injured trees resulted.

THE CHINQUAPIN BURN

Another area, which was studied in less detail than either of the others, is the Chinquapin burn, which is located 26 miles southeast of Ashland, Oreg. This fire occurred in September, 1915, and

covered 220 acres.   The character of this fire was practically the same as that on the Mistletoe and Siskiyou burns.

The attractive influence of the fire-injured trees on this burn was most marked during 1916, the year following the fire.   During this season there was a concentration of the western pine beetle within the area, resulting in an increase of 475 per cent over that on the area in 1915.   Most of the attacks were in trees which had been scorched by the fire.   While the infestation within the burn increased 475 per cent during this season, throughout the surrounding area it decreased 68 per cent.

In 1917 the infestation decreased both within the burn and in the surrounding area.   This was followed in 1918 by an increase both within the burn and the surrounding area.

On the whole, the data from this area indicate that the main effect of this fire, the concentration of beetles within the fire area, had disappeared by the close of the second season after the fire.

EFFECTS OF CONTROLLED BURNING UPON BARK-BEETLE INFESTATIONS

A fire of an entirely different character was studied in Siskiyou County, Calif., where a large lumber company during the season of 1920 practiced on its own holdings what has been termed "controlled" or "light" burning.   The method consisted of burning the area at night in small blocks of not more than 160 acres surrounded by fire lines.   During the period from June 15, 1920, to about October 30, 1920, approximately 12,000 acres were burned over by this system.   The object of this operation was to lessen the fire hazards within a tract where the company contemplated logging in the near future.

In character, the area differed essentially from those previously described in that there were few steep slopes, a very large part of the acreage being relatively flat.   Because of this and the fact that the fires were controlled and the burning was carried on at night, there was very little evidence of severe damage to merchantable trees such as occurred on the burns described.   The character of this fire also differed from the others in that the burning operations were extended over a considerable period of time.

Before the burning was started in 1920, representatives of the Bureau of Entomology made a 100 per cent cruise of one section of 640 acres, marking all insect-infested trees.   This section, which was among the first to be covered by the burning operations, was burned over in July.   One month later a second cruise was made of the same section.

These examinations yielded the following data:

	Number of trees
Trees infested at time of fire (first generation, 1920)	43
Brood mortality observed in infested trees exposed to fire	0
Number of standing trees attacked subsequent to fire	78
Number of trees burned down by fire in butt scars and subsequently attacked by beetles	12
Total loss on section during 1920 (year of fire)	133
Total loss on section during 1919 (year preceding fire)	79
Increase during 1920	54

It would appear from these figures that the fire caused some attraction which brought beetles into the area during the latter part of

the summer in which the fire occurred. However, no such concentration as that found on the other burns was observed. The failure of the beetles to concentrate on the light-burned area may have been due to the absence of trees sufficiently injured by the fire to attract the beetles, and also to the fact that for the remainder of the flight period of that season fires were running through near-by timber, creating conditions similar to those on the section that was cruised. This section was logged off the following spring, so that no follow-up studies were possible.

At the time that the burning operations were in progress the same company, under the technical advice of the Bureau of Entomology, organized a project for the control of the western pine beetle on a tract of 44,000 acres adjoining the 12,000 acres covered by controlled burning. From cruises made during the course of this project it was found that the loss during 1920 on the entire tract averaged 77 trees per section, or 112 board feet per acre, or 1.4 per cent of the total pine stand. Direct control measures against the beetles were started in the fall of 1920. The methods consisted of locating the infested trees, felling, and peeling and burning the infested bark. Two sections that had been burned over by the controlled burning of the summer extended into the area that was included in the insect-control work. As a 100-per cent cruise was made of these two sections and the adjoining area, it was possible to compare the overwintering infestation on the burned and unburned areas.

Number of infested trees per section on burned area_____ 189
Number of infested trees per section on adjoining unburned area_____ 170

It is evident that in this class of fire a very limited concentration of beetles resulted. Practically all of the area covered by the light-burning operations in 1920 was logged off during the following two seasons, so that it was possible to study only the results which developed immediately after the fire. As both control work and logging operations were carried on within the area subsequent to the burning, the natural tendencies of the infestation were considerably disturbed.

GENERAL SUMMARY

Forest fires of sufficient severity to scorch the bark, cambium, and foliage of yellow pine trees produce types of injury which make certain trees especially attractive to the western pine beetle. Many trees which have been only moderately injured by the fire and are apparently capable of recovering are attacked and killed by the beetles after a fire of this character.

The attraction of fire-injured trees often causes a concentration of beetles within a burned area which lasts for one or two seasons following the fire. This attraction may extend for a distance of 2 or 3 miles from the burn.

The concentration of bark-beetle attacks in fire-injured trees within a burned area does not develop into an epidemic condition. The loss from bark-beetle attacks in trees not injured by the fire either within the area of the burn or in the surrounding forest is not materially increased as a result of this concentration.

Fires which are not severe enough to burn the bark from infested trees are of little value in controlling bark-beetle infestations. Fires of moderate severity do not kill bark-beetle broods in infested trees that are on the area at the time of the fire.

While fire-injured trees attract bark beetles in considerable numbers they do not always afford favorable breeding conditions for the new broods. Where the trees have been defoliated by fire, an abnormally moist condition of the inner bark may follow, which results in high mortality of the broods in the early larval stages. Conditions have been observed where this mortality was so great as to act as a controlling influence, fewer beetles emerging from these trees than attacked them.

By and large, these studies show that fires can be of but little benefit in reducing beetle losses through killing the beetles unless the fires are severe enough to kill the trees; neither do fires have any permanent effect in increasing beetle losses in surrounding timber. The principal rôle of bark beetles in connection with forest fires is in the added destruction by the insects of moderately fire-injured trees, which otherwise would have survived.

Thus bark beetles supplement and increase the timber losses initiated by forest fires, while fires have but little influence in permanently increasing the losses caused by bark beetles.

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THE PARASITES OF THE PINE TIP MOTH, RHYACIONIA FRUSTRANA (COMSTOCK) [1]

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INTRODUCTION

The Nebraska National Forest is an entirely artificial plantation of pines of various species, situated in the sand hills just west of Halsey, Nebr. Unfortunately, with some of the young trees brought in for planting, certain pine-infesting insects were introduced into the forest. Among these was a tip moth, *Rhyacionia frustrana* var. *bushnelli* Busck, which has increased to such an extent that it has killed or badly stunted a very large percentage of the western yellow pines, the most favorable species for the development and increase of the pest.

In 1924 the Forest Service and the Bureau of Entomology instituted an attempt to control the insect by means of parasites.

To determine what eastern insects were available for introduction, the writer in 1924 made a preliminary study of the parasites of the eastern form of the tip moth, *Rhyacionia frustrana* (Comstock). Several thousand tips of *Pinus virginiana* infested by the summer generation of the tip moth were collected, principally in the vicinity of Falls Church, Va., and the insects contained in these were reared. A few parasitized tip worms were also collected in the spring of 1925. In the rearings were 21 species of parasitic Hymenoptera and two species of parasitic flies. A small lot of material collected at Bogalusa, La., by R. A. St. George, of the Bureau of Entomology, produced six species of parasitic Hymenoptera.

There had previously been recorded only three species of parasites of *Rhyacionia frustrana*. In 1890 Packard [2] reported as parasites a species of Bracon and a species of Perilampus, and in 1921 Cushman [3] listed this insect among the hosts of *Itoplectis conquisitor* (Say). In the meantime Carl Heinrich, then of the Division of Forest Insect Investigations, had reared some parasites from Virginia and Massachusetts; and a few other parasites of the species from other localities had been reared by members of the division.

All of these parasites are classified and discussed below on the basis of their relation to the host insect. A key for the identification of the parasites is appended.

EXTERNAL PARASITES OF LARVA

HYMENOPTERA

BETHYLIDAE

GONIOZUS LONGICEPS KIEFFER

Goniozus longiceps, originally described from Texas, has been reared in small numbers from tip-moth larvae from Bogalusa, La. It is probably gregarious on full-grown or nearly full-grown larvae.

ICHNEUMONIDAE

EPIURUS INDAGATOR (CRESSON)

Epiurus indagator is a common parasite of internally feeding microlepidopterous larvae, with many known hosts. It attacks full-grown or large larvae and does not confine its attack to insects infesting coniferous trees. Its distribution covers the eastern half of the United States and southern Canada. In the rearings of 1924, at Falls Church, it was rather rare. This species is solitary; the cocoon consists of a thin silken lining to the burrow of the host.

CALLIEPHIALTES COMSTOCKII (CRESSON)

Calliephialtes comstockii apparently confines its attack to lepidopterous larvae infesting coniferous trees, but prefers larger species than *frustrana*. It might do well on variety *bushnelli*, which is larger, but the rearings of 1924 at Falls Church on *frustrana* produced only a few undersized males. It is solitary on full-grown larvae. It spins a light brownish, semitransparent cocoon. Its distribution is about like that of Epiurus, except that it is confined to regions of coniferous forests.

BRACONIDAE

MICROBRACON MELLITOR (SAY)

This is a very common species, of catholic taste as regards hosts, attacking almost any internally feeding larva, whether lepidopterous or coleopterous. It is one of the principal parasites of the cotton boll weevil and of the pink bollworm. It is distributed throughout the eastern half of the country. In the 1924 Falls Church rearings from *frustrana* it was rare. It is solitary on full-grown or nearly full-grown larvae, and pupates in a white to brownish, opaque cocoon.

MICROBRACON GEMMAECOLA (CUSHMAN)

A few specimens of this species were reared at Falls Church in 1925 but none in 1924. In 1926 it was the most abundant parasite in Nantucket. It is very similar in its habits to *mellitor*.

MICROBRACON GELECHIAE (ASHMEAD)

Microbracon gelechiae is a gregarious parasite of internally feeding microlepidopterous larvae, from a few to several developing on a single host. It attacks large larvae. It occurs in the eastern half of the country and on the west coast. In the 1924 rearings at Falls Church it was very rare. It spins a dense brown cocoon.

EURYTOMIDAE

EURYTOMA TYLODERMATIS (ASHMEAD)

Eurytoma tylodermatis was the most abundant of all the parasites in the 1924 rearings at Falls Church. It is normally primary but occasionally secondary. It attacks large larvae, and is parasitic on many internally feeding lepidopterous and coleopterous larvae over at least the eastern half of the United States and southern Canada. The species is solitary.

EULOPHIDAE

SECODELLA SUBOPACA (GAHAN)

This is a solitary parasite of small larvae. There were a few females in the 1924 rearings at Falls Church and a single male from Bogalusa, La.

HYSSOPUS THYMUS (GIRAULT)

Hyssopus thymus is a minute gregarious parasite of full-grown or nearly full-grown larvae, from 3 to 10 or 12 developing on a single host. It was rather rare in the 1924 and 1925 rearings at Falls Church. It was originally described from Lincoln, Nebr.

HYSSOPUS RHYACIONIAE (GAHAN)

Hyssopus rhyacioniae is exactly similar in habit to *H. thymus*, but is less common. It was reared at Falls Church in 1924.

ELACHERTUS PINI GAHAN

This is another gregarious parasite, similar in its host relations to the species of Hyssopus, but less abundant. It was reared only at Falls Church.

PTEROMALIDAE

HABROCYTUS THYRIDOPTERIGIS HOWARD

Habrocytus thyridopterigis was reared sparingly at Falls Church in 1924 as a solitary primary parasite and in 1925 as a primary and in one case as a secondary parasite, the intermediate host being *Campoplex frustranae*, a cocoon of which produced five males of Habrocytus. In 1916 *H. thyridopterigis* was reared at Falls Church by Heinrich, and in that year it was apparently one of the most abundant parasites of the tip moth. Heretofore it has been recorded only as a secondary parasite of the bagworm, *Thyridopteryx ephemeraeformis* Haworth, and the white-marked tussock moth, *Hemerocampa leucostigma* Smith and Abbot.

INTERNAL PARASITES OF LARVA

HYMENOPTERA

ICHNEUMONIDAE

COMPOPLEX FRUSTRANAE CUSHMAN

Next to *Eurytoma tylodermatis*, *Campoplex frustranae* was the most abundant parasite in the 1924 rearings at Falls Church. It attacks its host probably when the latter is comparatively young, and spins its white cocoon within the shattered shell of the newly formed pupa of the host.

CREMASTUS EPAGOGES CUSHMAN

This species was of rare occurrence in the 1924 rearings at Falls Church. In habit it is similar to Campoplex, but leaves its host while the latter is still in the larval stage and spins a dense dark-brown cocoon. It was originally described from specimens reared at Nashville, Tenn., from *Epagoge sulfureana* Clemens.

GLYPTA VARIPES CRESSON

Glypta varipes was reared from the tip moth only at Sharon Heights, Mass., in 1916, and at Nantucket, Mass., in 1926, where it appears to have been one of the more important parasites. This species is solitary.

BRACONIDAE

PHANEROTOMA RHYACIONIAE CUSHMAN

Phanerotoma rhyacioniae is known only from the tip moth and only from Bogalusa, La., where, judging from the small amount of material available, it was apparently the most important parasite. No observations on its life history have been reported, but probably it deposits its egg in the egg of the tip moth, its larva leaving the host when the latter is full-grown and pupating in a cocoon in the burrow of the host. It is probably solitary.

APANTELES SPECIES

There was a single specimen of Apanteles from Bogalusa, La.; it probably attacks the very young larva, leaving its host when the latter is nearly full-grown.

DIPTERA

TACHINIDAE

LIXOPHAGA MEDIOCRIS ALDRICH

The third most abundant parasite in the 1924 rearings at Falls Church was *Lixophaga mediocris*. The full-grown larva emerges from the larva of the host and pupates in the host burrow. It was described as a new species from these specimens.

LIXOPHAGA PLUMBEA ALDRICH

This is another new species with habits exactly similar to those of *Lixophaga mediocris*. It was much less abundant than the latter species at Falls Church in 1924.

PARASITES OF PUPA

HYMENOPTERA

ICHNEUMONIDAE

ITOPLECTIS CONQUISITOR (SAY)

This is one of the most common Ichneumonidae throughout the eastern half of the United States, southern Canada, and the West Indies, parasitizing lepidopterous pupae of nearly all groups. It is rare as a parasite of the tip moth, which is too small to be a favorable host; only a few undersized males were reared. It pupates within the host pupa.

CHALCIDIDAE

HALTICHELLA RHYACIONIAE GAHAN

Of all the parasites in the 1924 rearings at Falls Church, *Haltichella rhyacioniae* ranked fourth in point of abundance, and it was also one of the most abundant in Heinrich's 1916 rearings. There was one specimen from Nantucket, Mass. This species pupates in the host pupa.

SECONDARY PARASITES AND THOSE OF UNKNOWN STATUS

Under this head are placed seven species of chalcidoids and one braconid, all reared in small numbers, on which no definite observations for determining their status were made. The first three, because of the known habits of their congeners, are almost certainly secondaries.

HYMENOPTERA

CHALCIDIDAE

SPILOCHALCIS DELIRA (CRESSON)

SPILOCHALCIS SPECIES

These are secondarily parasitic through pupal parasites.

PERILAMPIDAE

PERILAMPUS SPECIES

EUPELMIDAE

EUPELMUS CYANICEPS ASHMEAD

Eupelmus cyaniceps is a ubiquitous species of very wide distribution and catholic taste in the matter of hosts. It attacks Lepidoptera, Coleoptera, and Hymenoptera, sometimes as a primary parasite and sometimes as a secondary parasite. Its exact relationship to the tip moth is unknown, but very likely it is both a primary and a secondary parasite.

EULOPHIDAE

EPITETRASTICHUS CUNEIFORMIS GIRAULT

This species was reared in small numbers at Falls Church in 1924 and by Heinrich in 1916. It is probably not associated in any way with the tip moth, but rather with some species of midge infesting the tips. It was originally reared in such an association.

TETRASTICHUS SPECIES

There were two specimens from the Bogalusa, La., material.

ELASMIDAE

ELASMUS SETOSISCUTELLATUS CRAWFORD

One specimen of this species was reared at Falls Church in 1924, probably from a midge, such an insect having been the host in a previous rearing.

BRACONIDAE

HETEROSPILUS SPECIES

A single specimen of Heterospilus was reared from infested tips at Nantucket, Mass. Most likely Heterospilus is not parasitic on the tip moth, but in this instance was associated with some coleopterous borer.

HOW TO RECOGNIZE THE PARASITES

The following key will serve to identify the parasites listed above:

1. With one pair of wings and having the general appearance of a small
    house fly (Diptera)____*Lixophaga mediocris* Aldrich and *Lixophaga plumbea*
                        Aldrich. (These two flies are very similar in
                        appearance and distinguishable only to the
                        trained eye.)
    With two pairs of wings and more wasplike in appearance, some very
        minute (Hymenoptera)_____ 2
2. Wings with many veins (fig. 1, *a–e*)_____ 3
    Wings with veins only along front margin (Chalcidoidea) (fig. 1, *f*)___ 15
3. Antennae very short and placed close to mouth; eyes very small
        (fig. 1, *h*); wings as in figure 1, *a* (Bethylidae)___*Goniozus longiceps* Kieffer
    Antennae long and slender and placed far above mouth; eyes large
        (fig. 1, *g, i*)_____ 4
4. Front wing about as in Figure 1, *b* or *c* (Ichneumonidae)_____ 5
    Front wing about as in Figure 1, *d* or *e* (Braconidae)_____ 10
5. Abdomen flattened below, rounded above (fig. 1, *j*), the first segment
        broad at the base_____ 6
    Abdomen compressed from side to side, the first segment long and
        very narrow at base (fig. 1, *k*)_____ 9
6. Abdomen with segments edged above with whitish or yellowish
                        Itoplectis conquisitor (Say)
    Abdomen with segments entirely black above_____ 7
7. Middle segments of abdomen with deep oblique furrows extending
        from middle of base toward hind angles (fig. 1, *j*)___*Glypta varipes* Cresson
    Middle segments of abdomen without oblique furrows_____ 8
8. Hind tibia with alternate rings of white and black
                        Epiurus indagator (Cresson)
    Hind tibia white only at base_____*Calliephialtes comstockii* (Cresson)
9. Head entirely black; front wing with the small cell marked *a* in Fig-
        ure 1, *b*_____*Campoplex frustranae* Cushman
    Head with yellow rings surrounding the eyes; front wing as in Figure
        1, *c*, without cell *a*_____*Cremastus epagoges* Cushman
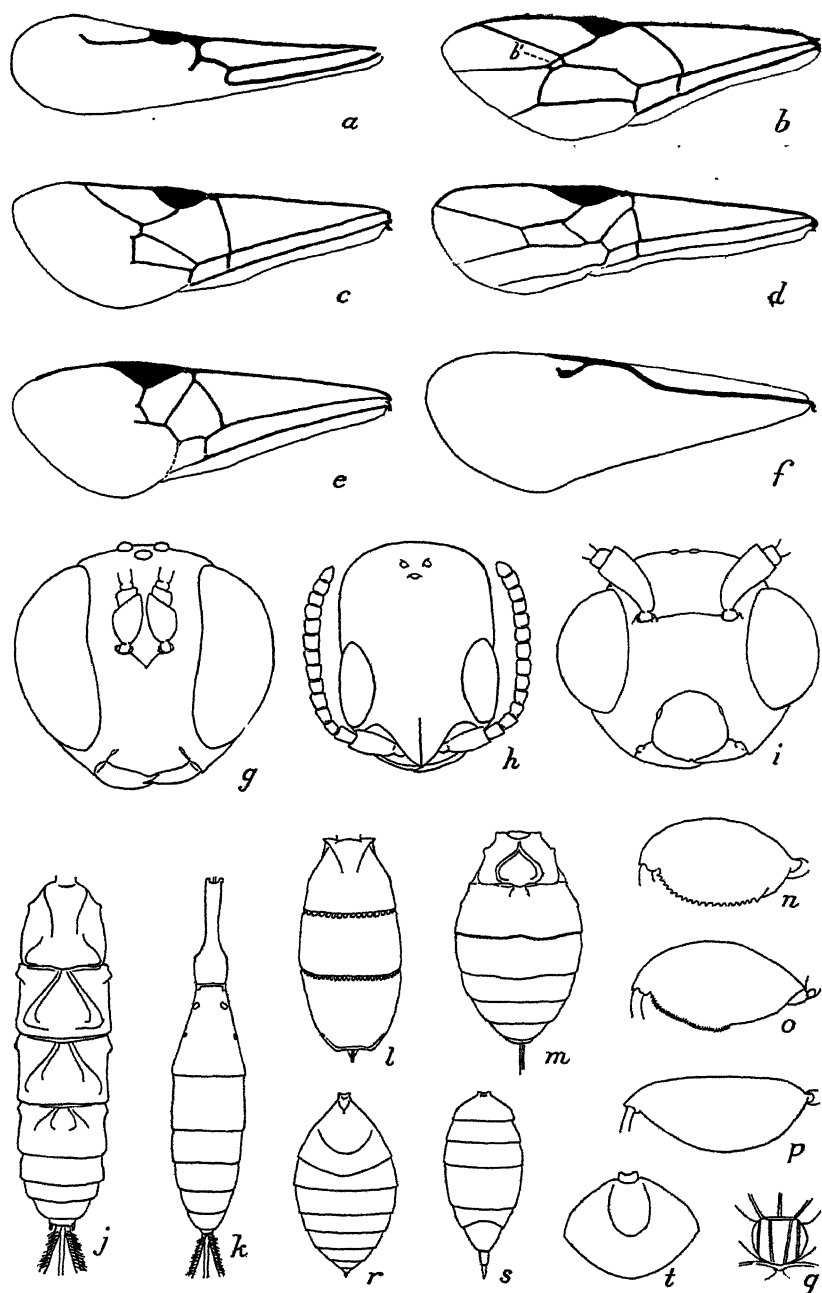
Fig. 1.—*a*, Forewing of *Goniozus longiceps* Kieffer; *b*, forewing of *Epiurus indagator* (Cresson): *c*, forewing of *Cremastus epagoges* Cushman; *d*, forewing of *Microbracon gemmaecola* Cushman; *e*, forewing of *Apanteles* species; *f*, forewing of *Eurytoma tylodermatis* Ashmead; *g*, head of *Campoplex frustranae* Cushman; *h*, head of *Goniozus longiceps* Kieffer; *i*, head of *Phanerotoma rhyacioniae* Cushman; *j*, abdomen of *Glypta varipes* Cresson; *k*, abdomen of *Campoplex frustranae* Cushman; *l*, abdomen of *Phanerotoma rhyacioniae* Cushman; *m*, abdomen of *Microbracon gemmaecola* Cushman; *n*, hind femur of *Spilochalcis delira* (Cresson); *o*, hind femur of *Haltichella rhyacioniae* Gahan; *p*, hind femur of *Elasmus setosiscutellatus* Crawford; *q*, scutellum of *Epitetrastichus cuneiformis* Girault; *r*, abdomen of *Hyssopus thymus* Girault; *s*, abdomen of *Eurytoma tylodermatis* Ashmead; *t*, abdomen of *Perilampus* species

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10. Wings as in Figure 1, *c*; body entirely black___ __ __ _ _ _ *Apanteles* sp.
    Wings more similar to Figure 1, *d*; body usually more or less yellowish
    or reddish_ 11
11. Abdomen with only three visible segments (fig. 1, *l*)
                                        Phanerotoma rhyacioniae Cushman
    Abdomen with more than three visible segments (fig. 1, *m*)_ _ _ _ _ _ _ 12
12. Largely blackish_ *Microbracon gelechiae* Ashmead
    Largely reddish yellow_ 13
13. First segment of abdomen with a triangular area set off by deep
    grooves as in Figure 1, *m*_ 14
    First segment of abdomen without such an area_ _ _ _ _ _ _ *Heterospilus* sp.
14. Wings distinctly smoky; ovipositor fully as long as abdomen
                                        Microbracon mellitor (Say)
    Wings clear; ovipositor shorter than abdomen
                                        Microbracon gemmaecola Cushman
15. Hind femur very deep (fig. 1, *n–p*)_ 16
    Hind femur not especially deep_ 19
16. Body entirely black_ 17
    Body more or less spotted with yellow_ 18
17. Body and legs strongly flattened from side to side and having some-
    what the appearance of a winged flea; minute
                                        Elasmus setosiscutellatus Crawford
    Body stout, not flattened from side to side_ _ _ _ *Haltichella rhyacioniae* Gahan
18. Abdomen largely reddish with several yellow spots
                                        Spilochalcis delira (Cresson)
    Abdomen black with two yellow spots_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ *Spilochalcis* sp.
19. Bright metallic green, blue, or bronze_ 20
    Black with at most faint metallic luster_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 22
20. Blue; very minute_ *Secodella subopaca* Gahan
    Green or bronze_ 21
21. Body entirely bright green; ovipositor (the sting) not protruding
                                        Habrocytus thyridopterigis Howard
    Not entirely bright green; ovipositor protruding and ringed with
    yellow_ *Eupelmus cyaniceps* (Ashmead) (female)
22. Dark blue_ *Eupelmus cyaniceps* (Ashmead) (male)
    Black or black and brown_ 23
23. Scutellum with four longitudinal grooves (fig. 1, *q*)_ _ _ _ _ _ _ _ _ _ _ _ 24
    Scutellum with at most two longitudinal grooves_ _ _ _ _ _ _ _ _ _ _ _ _ _ 25
24. Head more or less brownish_ _ _ _ _ _ _ _ _ _ _ _ *Epitetrastichus cuneiformis* Girault
    Head black_ *Tetrastichus* sp.
25. Abdomen very broadly oval, much broader than deep (fig. 1, *r*.; very
    minute_ 26
    Abdomen not broadly oval above, lanceolate or broadly triangular
    (fig. 1, *s*, *t*)_ 28
26. Abdomen with a whitish spot at base_ _ _ _ _ _ _ _ _ _ _ _ *Elachertus pini* Gahan
    Abdomen entirely black_ 27
27. Thorax prolonged in front so that the head appears to be set out on a
    neck_ *Hyssopus rhyacioniae* Gahan
    Thorax not prolonged in front_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ *Hyssopus thymus* Girault
28. Abdomen flattened from side to side (fig. 1, *s*); joints of antennae in
    male beadlike, with long hair_ _ _ _ _ _ _ _ _ _ *Eurytoma tylodermatis* Ashmead
    Abdomen triangular (fig. 1, *t*); antennae in male not as above
                                        Perilampus sp.

ADAPTATION OF THE BACTERICIDAL ACTION OF CHLO-ROFORM TO THE PREPARATION OF BACTERINS [1]

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REVIEW OF RELATED WORK

It is not the purpose of this paper to herald a discovery, but rather to call attention to an unusual, but practical, application of facts already at hand relating to the killing of vegetative bacteria by exposing them to bactericidal vapors, especially to that of chloroform. (*3, p. 166.*) [2]

Chloroform has long been widely accepted as a preservative. When it is added to immune sera (*5, p. 667*), the reason is obviously to check contaminating growth, rather than to "kill off" a specific organism in the fluid, as in the case of bacteries.

It has long been known (*1, p. 117*) that, although chloroform can inhibit the activity of microorganisms, it has no effect on their enzymes. On the other hand, the destructive effect of even moderate heat upon the products of bacteria has been generally emphasized. Hiss and Zinsser (*2, p. 43, 168*) assert that—

temperatures of over 70° C. permanently destroy most enzymes. * * * Against the weaker disinfectants in common use, enzymes often show a higher resistance than do the bacteria which give rise to them. * * * Both of these substances [chloroform and toluol] will destroy the bacteria without injuring the enzymes.

In discussing the preparation of vaccines, Kolmer (*4, p. 657*) says:

The specific microorganism or virus used in a vaccine should be modified as little as possible, or just sufficient to rob it of its disease-producing power. * * * The less modification the better the vaccine. If the exposure [to heat] is too prolonged or the temperature too high, the vaccinogenic power of the bacilli is destroyed, and the suspension in salt solution is no more potent or of no greater value than the salt solution itself. Therefore the nearer the vaccine approaches the fully viable virus or microorganism, the more potent it will be.

The known destructive action of heat upon the products of bacteria as compared with the lack of such action exerted upon these products by chloroform would appear, in the light of Kolmer's statement, to be a fair criterion of the conversely relative degrees of potency of bacteries made by these respective methods. Unpublished data, accumulated by the writer in connection with extensive experiments on the biological therapy of bovine mammitis, have shown clearly the greater potency of autogenous bacteries treated with chloroform as compared with those treated with heat.

These findings are in harmony with those of Vincent and Collignon (*6*), who observed that certain bacterial suspensions could be killed

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Journal of Agricultural Research,   Vol. 34, No. 7
Washington, D. C.   Apr. 1, 1927
Key No. A-116

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by direct exposure to ether, and could be employed advantageously for immunization. This method is discussed by them in part as follows:

The antigen employed was prepared according to the principle which one of us had made known for the preparation of antityphoid serum. The cultures of the micrococcus (Binot), three days old on gelatin, were emulsified in physiological salt solution (10 c. c. for a large tube of culture), after which ether was added. The mixture, vigorously shaken for one or two minutes, was carefully corked up, and then left for 24 hours at laboratory temperature. After this interval the ether was evaporated out at a temperature of 38° for a few minutes. This culture, thus killed, was employed as a vaccine * * *.

It was hoped that we might be able to obtain a practical immunization of the goat against Malta fever by several subcutaneous injections of cultures of the microbe of that disease, sterilized by ether, which was a volatile antiseptic, easily manipulated and easily disposed of. Thus treated, the virus lost none of its immunizing properties and promoted the production of antibodies almost as energetically as would a living culture. For the purpose of immunizing animals, this antigen is far preferable to a living attenuated culture of the *M. melitensis*, because the living culture, even though it is attenuated, might cause the animals to become disseminators of the germ. It was equally superior to cultures killed by heat.

TESTS WITH CHLOROFORM—SUSPENSION METHOD

In the laboratory of the Pathological Division the need of an effective method of sterilizing bacterial suspensions without heat was recognized. In some instances the use of serum broth for the propagation of the bacteria was attended with good results until the process reached the stage of sterilization by heat. During this operation, however, the serum in the medium became coagulated and clouded the product, to its obvious detriment.

Out of this exigency was conceived the idea of sterilization by other means than heat. The use of certain chemicals offered a possible solution of the problem. Chloroform was first employed in the following manner: Beef-infusion broth, containing about 1 per cent of normal beef serum, was sterilized by passage through a bacteria-retaining filter. This broth was distributed into sterile 200 c. c. Erlenmeyer flasks, 100 c. c. of broth to each flask. Sterility was tested and proved by inoculation.

Six of these flasks were then inoculated with a culture of *Staphylococcus aureus* and incubated 24 hours. Test cultures were made on agar slants and heavy growths resulted. Meanwhile the cotton plugs were removed from the flasks, rubber stoppers were substituted, and a small homeopathic vial was suspended by a slender wire in each flask (fig. 1). One cubic centimeter of chloroform was placed in each vial immediately before the vials were inserted in the flasks. The flasks were left at room temperature, which was then noted to be 79° F. Variation from this temperature was so slight as to be negligible during the course of the experiment.

At the expiration of four hours after the suspension of the chloroform vials within the flasks test cultures were made on agar from each flask, and all were found to contain the living organism. Again after the expiration of 24 hours following the suspension of the vials, a second set of tests of cultures was made. Twenty-four hours' incubation proved them all to be sterile. At this time, exactly 48 hours after the commencement of the experiment, it was observed that the chloroform had entirely evaporated from within the vials. The

vials were then aseptically removed, and the stoppers replaced. The flasks were left at room temperature for a period of six days and three hours, after which cultures were made in agar to confirm the sterility of the suspensions. During the six-day period the rubber stopper had inadvertently become dislodged from flask No. 5, and the broth in this flask had become contaminated. With this exception, all the flasks of broth culture proved to be still sterile.

To test quantitatively the amount of chloroform required to kill 100 c. c. of the serum-broth culture, a second series of tests was made.

Six flasks were inoculated with *Pseudomonas pyocyanea*, and incubated. Test cultures showed the presence of living organisms in all flasks.

After five days the plugs were aseptically removed, as before, and the rubber stoppers and suspended vials containing chloroform were substituted. Different amounts of chloroform were employed, as follows: Flask No. 1, 0.3 c. c.; No. 2, 0.5 c. c.; No. 3, 1.0 c. c.; No. 4, 3.0 c. c.; No. 5, 5.0 c. c.; and No. 6, viability control, none.

After a lapse of 2 hours and 50 minutes, and again of 4 hours and 30 minutes, test cultures from all six flasks were made on agar, which proved by incubation to contain the organism in every instance.

After a total lapse of 21½ hours another set of test cultures was made. All proved to be sterile except that in flask

Fig. 1.—Apparatus for sterilizing cultures with vial containing chloroform: *a*, Rubber stopper; *b*, small vial suspended from stopper, containing chloroform; *c*, broth culture of living organisms

No. 6. This flask had received no chloroform fumigation, and now contained a heavy suspension of viable organisms.

At this time observation was taken on the quantity of chloroform remaining in the vials. The observations, roughly tabulated, are as follows: Flask No. 1, chloroform completely evaporated; No. 2, completely evaporated; No. 3, nearly all evaporated; No. 4, nearly all present; No. 5, nearly all present.

It was thus demonstrated that as low a proportion as 0.3 per cent of chloroform would sterilize a suspension of *Pseudomonas pyocyanea* in 21½ hours at incubator temperature.

POTENCY TESTS OF CHLOROFORM AND OTHER CHEMICALS

An experiment was instituted to test the relative bactericidal potency of several volatile chemicals in different proportions, against a nonspore-bearing aerobe.

With *Pasteurella boviseptica*, eight flasks of broth (100 c. c. each) were inoculated March 14 at 4 p. m. and placed in the incubator.

On March 20 at 10 a. m. they were removed from the incubator, and at 3 p. m. chemicals were suspended in flasks as follows:

Flask No. 1, chloroform, 1 c. c.: flask No. 2, chloroform, 0.1 c. c.; flask No. 3, formaldehyde solution United States Pharmacopoeia, 1 c. c.; flask No. 4, formaldehyde solution, United States Pharmacopoeia, 0.1 c. c.; flask No. 5, ammonia fortior, 1 c. c.; flask No. 6, ether, 1 c. c.: flask No. 7, ether, 0.1 c. c.; flask No. 8, control—no chemical.

Test agar-slant cultures (a) made at this time proved to contain living *Pasteurella boriseptica* organisms. No. 5, however, was contaminated.

On March 21 at 11 a. m. (20 hours later), test agar-slant cultures (b) were made from each flask, which upon incubation gave the following results:

Culture No. 1–b, sterile; culture No. 2–b, growth of *Pasteurella boriseptica* plus contaminating mold; culture No. 3–b, growth of *P. boriseptica*; culture No. 4–b, growth of *P. boriseptica;* culture No. 5–b, growth of *P. boriseptica;* culture No. 6–b, growth of *P. boriseptica;* culture No. 7–b, growth of *P. boriseptica;* culture No. 8–b, growth of *P. boriseptica*.

On March 23 at 11 a. m. (68 hours after beginning fumigation) test agar-slant cultures (c) were made from each flask, with the following results:

Culture No. 1–c, sterile; culture No. 2–c, growth of *Pasteurella boriseptica;* culture No. 3–c, sterile; culture No. 4–c, sterile; culture No. 5–c, growth of *P. boriseptica;* culture No. 6–c, sterile; culture No. 7–c, growth of *P. boriseptica·* culture No. 8–c, growth of *P. boriseptica* (control).

It is apparent that formaldehyde solution U. S. P. kills in the same and even in smaller quantities than chloroform, but the latter is more desirable in that its action is more rapid. This may be attributable to the fact that chloroform vapor, being heavier than air, concentrates more fully upon the exposed surface of the culture.

Ether vapor, although effective in its bactericidal action, is undesirable for practical use because of its high inflammability.

CHLOROFORM TESTS WITH VARIOUS ORGANISMS

At the conclusion of these experiments various organisms were tested for susceptibility to sterilization by chloroform fumigation such as had proved effective for *Staphylococcus aureus, Pseudomonas pyocyanea,* and *Pasteurella boriseptica.*

The following nonspore-bearing organisms were implanted each in a 200 c. c. flask, containing 100 c. c. of plain infusion bouillon: *Aerobacter cloacae, Alcaligines abortus, A. bronchisepticus, A. melitensis, Chromobacterium violaceum, Corynebacterium ovis, Eberthella sanguinarium, Erysipelothrix rhusiopathiae, Erythrobacillus prodigiosus, Escherichia coli* (swine origin), *Hemophilus pyogenes, Pasteurella boriseptica, Salmonella abortivo-equina, S. aertrycke, S. paratyphi, S. pullorum, S. suipestifer, Staphylococcus aureus, S. citreus,* Streptococcus (canine).

These cultures were placed in an incubator and removed after 46 hours' incubation. Control cultures (series A) were made and 1 c. c. of chloroform was then suspended in each flask in the manner

previously described. All the series A control cultures had a good growth after 24 hours' incubation except *Hemophilus pyogenes*, *Alcaligines abortus*, and *A. melitensis*. Since these are very delicate and slow-growing organisms, they were continued in the experiment, as it was thought that longer incubation might establish the viability of the cultures. After an additional 24 hours' incubation at room temperature an appreciable growth of *A. abortus* and *A. melitensis* was noted, but the culture of *H. pyogenes* remained sterile, and was therefore eliminated as having been a dead culture from the beginning of the experiment.

Control cultures (series B) were made after the broth cultures had been subjected to chloroform fumigation for 24 hours. At this time the vials were removed and the rubber stoppers replaced.

After 24 hours' incubation these controls were found to be sterile except for a few colonies on culture tube No. 15 (Streptococcus [canine]) and tube No. 37 (*Aerobacter cloacae*). It was thought possible that the stoppers had been left sufficiently loose to permit the escape of the chloroform fumes before their bactericidal action had taken place. New flasks of broth were therefore inoculated with each of these organisms and this part of the experiment repeated. A tube of serum agar was also inoculated from each of the original flasks.

After four hours' incubation, test cultures of these two organisms (series A) were made from each flask on to serum agar, and then 1 c. c. of chloroform was suspended in each flask, rubber stoppers inserted, and all were left at room temperature. Upon incubation, a luxuriant growth was obtained of all test cultures (series A).

The chloroform vials were removed after 24 hours and test cultures (series B) were made on agar slants. These were incubated for 24 hours, at the end of which time they were all found to contain typical growths. It was concluded, therefore, that this method of sterilization was not effective in killing very resistant nonspore-bearing aerobes. It should be added, however, that the writer has repeatedly employed this method for killing streptococci of diverse origin, with entirely satisfactory results, indicating that the streptococcus employed as culture No. 15 must have possessed extraordinary qualities of resistance.

Three spore-bearing aerobes were planted in separate flasks of 100 c. c. of broth each, preparatory to determining what action, if any, chloroform fumigation would exert on this class of bacteria. The organisms used were *Bacillus megatherium*, *B. X*, and *B. vulgatus*. After 20 hours, test agar cultures (series A) were made from each flask, and 1 c. c. of chloroform was then suspended in each flask. Rubber stoppers were inserted instead of the cotton plugs, and all flasks were left at room temperature. All the test cultures proved to contain the viable organisms.

After 24 hours the chloroform vials were removed and test cultures series B) were made on agar slants. These were incubated for 24 hours, after which they were found to contain luxuriant growths of the organisms. It was, therefore, obvious that chloroform fumigation as here practiced would not be applicable to the sterilization of cultures of these spore-bearing aerobes.

TESTS WITH CHLOROFORM VAPOR

At the suggestion of John S. Buckley, chief of the Pathological Division, the writer directed his attention to the possibility of accomplishing the sterilization of fluid suspensions of organisms by the passage of chloroform vapor through them by means of air pressure or vacuum.

For this purpose an apparatus was developed, as illustrated in Figure 2, consisting of an Erlenmeyer flask, *a*, and a graduated
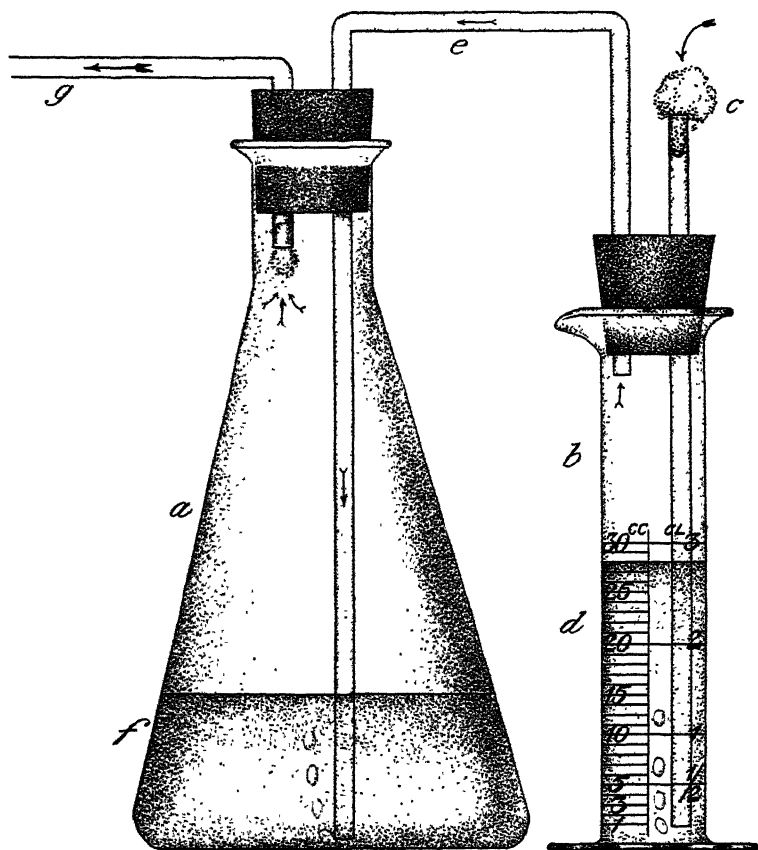
Fig. 2.—Apparatus for sterilizing with chloroform vapor: *a*, Erlenmeyer flask; *b*, graduated cylinder; *c*, inlet tube; *d*, column of chloroform; *e*, connecting tube; *f*, broth culture; *g*, vacuum connection

cylinder, *b*, both equipped with rubber stoppers fitted with an inlet and an outlet glass tube. Tube *c*, the inlet tube for the graduated cylinder, has inserted in its upper extremity a loose plug of cotton wool for the filtration of the air. Passing through this tube, the filtered air is drawn through a column of chloroform, *d*, of known volume, and then the air, now surcharged with the chloroform vapor, is carried through the bent, connecting tube *e* (which comprises the outlet tube of the graduated cylinder and the inlet tube of the Erlenmeyer flask) from the air space of the graduated cylinder, *b*,

to the depths of the broth culture, *f*.   As the chloroform vapor passes upward through the broth culture, some part of it becomes absorbed in the medium, and the residue, together with the air, escapes through the vacuum connection, *g*, which is fitted with a cotton plug within the flask, *a*.

The following experiments in sterilization with streaming chloroform vapor were performed at room temperature.   A series of agar cultures (series A) was made from three 500 c. c. flasks, each containing 100 c. c. of a broth culture of *Staphylococcus aureus*.   These agar cultures developed luxuriant growths of the organism.

By the use of the apparatus just described, flask No. 1, containing 100 c. c. of the *Staphylococcus aureus* broth culture, was subjected to a current of streaming air charged with the vapor of chloroform.   The amount of vacuum employed was not measured, but was such as to set up a lively ebullition in the chloroform, and also in the culture flask.   Care was taken, however, that this bubbling activity should not become so violent as to carry over drops of liquid chloroform into the culture.   After this process had continued for 10 minutes the column of chloroform was measured, and it was observed by comparison with the original measure of the column that 2.5 c. c. of chloroform had been vaporized and passed through the broth culture.   This culture was then seeded upon agar (series B) and this, when incubated, yielded a heavy growth of *S. aureus*.

A similar process was carried out with a second flask (No. 2), containing a like culture.   This time, however, the ebullition was less violent and was continued for 30 minutes.   The quantity of chloroform converted into vapor and passed in this condition through the culture was 5 c. c.   Series B cultures from this flask likewise yielded a heavy growth of the organism.

At the end of 24 hours a third set of agar cultures (series C) was made from flasks Nos. 1 and 2, and these, upon incubation, showed only a sparse, scattered development of colonies, indicating that a decided bactericidal action had taken place in both flasks of broth culture after the previous test cultures had been made.   This bactericidal action was obviously due to the amount of chloroform which had been held in solution by the broth cultures.   The prolonged preservative effect of such retained chloroform renders it unnecessary to add phenol as a preservative to cultures promptly sealed after being killed with chloroform.

A third test was carried out under conditions similar to those in the previous tests except that by the reduction of the vacuum to a minimum the period of ebullition was increased to 2 hours and 45 minutes, and the quantity of chloroform used was 4.5 c. c.   The series B cultures from the third test proved to be sterile.

The advantage of this method over the chloroform-suspension method described earlier in this paper is that it accomplished the sterilization of the culture in a few hours instead of a day.   Thus, in the routine employment of this method, practically a day is gained in the time required to prepare a bacterin for use.   In the preparation of autogenous bacterins it is quite conceivable that this saving of a day might be a matter of the utmost importance to the patient.

It might be added that the quantity of chloroform retained in suspension in the bacterin is inconsequential so far as any detrimental

effects upon the patient is concerned. In the numerous injections of chloroform-killed suspensions into cattle and poultry performed by the writer, or under his direction, not the slightest local or systemic effect of chloroform has ever been noted.

SUMMARY AND CONCLUSIONS

Heat is destructive to the products of bacteria. The use of heat in the preparation of bacterins therefore reduces their potency.

Chloroform kills many nonspore-bearing bacteria with no important modification of their biochemical characteristics. The use of chloroform in the preparation of bacterins therefore conserves their potency. The preparation of killed cultures of spore-bearing organisms, and of nonspore-bearing organisms of resistant types is, however, not practicable by this method.

Chemicals such as formaldehyde solution U. S. P., ether, and possibly toluol, might be employed in the preparation of bacterins, but all are open to some objections.

The passage of 2.5 per cent of choloroform in streaming vapor through a broth culture of *Staphylococcus aureus* will not sterilize it in 10 minutes.

The passage of 5.0 per cent of choloroform in streaming vapor through a broth culture of *Staphylococcus aureus* will not sterilize it in 30 minutes.

The passage of 4.5 per cent of choloroform in streaming vapor through a broth culture of *Staphylococcus aureus* will sterilize the culture, provided the process is so retarded as to utilize that amount of chloroform, in a period of 2 hours and 45 minutes. It is important not only to pass an adequate quantity of vapor through the culture, but to retard the passage sufficiently to permit of maximum absorption of the vapor by the medium.

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THE NATURAL REPLACEMENT OF BLIGHT-KILLED CHESTNUT IN THE HARDWOOD FORESTS OF THE NORTHEAST [1]

By C. F. KORSTIAN, *Associate Silviculturist, Appalachian Forest Experiment Station*, and PAUL W. STICKEL, *Assistant Silviculturist, Northeastern Forest Experiment Station, Forest Service, United States Department of Agriculture* [2]

INTRODUCTION

Chestnut (*Castanea dentata*) once formed almost half the second-growth sprout hardwood forests of southern New England, New Jersey, and central and southern Pennsylvania; on small areas it often formed 90 per cent of the total wood volume. Its destruction by the chestnut blight (*Endothia parasitica*) has radically changed the composition of these forests. The rapid growth, abundance, and prodigious sprouting capacity of chestnut, together with its intrinsic value for a wide variety of uses, made it the most promising of the northeastern forest trees until the rapid spread of the blight made its extinction inevitable (*12*).[3]

The total loss of such a species naturally introduces serious problems as to the future silvicultural management of the forests of which it was an important component. These problems apply both to the North, where the chestnut is already dead, and to those parts of the Southern Appalachian region where chestnut still furnishes abundant and valuable forest products, but where it is already doomed (*4, 5, 13*).

Knowledge of what is happening on blight-killed chestnut areas is necessary in order to appraise the future growth rate of the changed forests and to determine the period of restoration of these lands to their full productive capacity with species other than chestnut.

Studies of the effects of the blight upon forests containing chestnut and upon the character and amount of natural replacement were undertaken as a first step toward supplying such information.

RESULTS OF 1920 STUDY OF CHESTNUT REPLACEMENT

The first field study of the natural replacement of chestnut by other species was made by the Forest Service, United States Department of Agriculture, in 1920, in western Virginia, Maryland, eastern Pennsylvania, and northern New Jersey.[4] Advance growth of desirable species sufficient to restock the stands was generally found. Larger-sized reproduction was not always present—thereby sometimes delaying future merchantable stands—although there were no vacant areas of any appreciable extent after the chestnut died. In some

Journal of Agricultural Research
Washington, D. C.

Vol. 34, No. 7
Apr. 1, 1927
Key No. F-38

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stands, especially on the more favorable sites, advance reproduction was inadequate to restock the area at once. This, together with the presence of dense underbrush, suggested the possibility that the regeneration of desirable species might be long delayed, even if the value of the future forest were not permanently impaired.

OBJECT OF 1924 STUDIES

The 1920 study was followed in 1924 by somewhat similar but more intensive field studies of blight-depleted stands in the regions of earlier infection in southern New England and New Jersey. In addition, limited studies were made in Pennsylvania of the progress of replacement. The 1924 studies were extended to cover representative stands of different ages on the more important chestnut sites.

The primary object of these studies was to determine the progress of the natural replacement of the chestnut by other species, and to forecast as nearly as possible the impending changes in the composition of the stand.

In these studies the stands were examined for restocking and were then analyzed to determine the extent to which the replacing species are silviculturally desirable and economically valuable. These species were accordingly arranged in three classes.

Class 1—Desirable species: Red oak (*Quercus borealis*); white oak, (*Quercus alba*): black oak (*Quercus velutina*); chestnut oak (*Quercus montana*); hickory (*Hicoria* spp.): white ash (*Fraxinus americana*); sugar maple (*Acer saccharum*): sweet birch (*Betula lenta*); black cherry (*Prunus serotina*); yellow poplar (*Liriodendron tulipifera*); basswood (*Tilia glabra*); northern white pine (*Pinus strobus*); pitch pine [5] (*Pinus rigida*).

Class 2—Less desirable species: Scarlet oak (*Quercus coccinea*); red maple (*Acer rubrum*); beech (*Fagus grandifolia*); black gum (*Nyssa sylvatica*); aspen (*Populus tremuloides*); largetooth aspen (*Populus grandidentata*); eastern cottonwood (*Populus deltoides*).

Class 3—Undesirable species:[6] Dogwood (*Cornus* spp.); gray birch (*Betula populifolia*): bear or scrub oak (*Quercus ilicifolia*); sassafras (*Sassafras variifolium*); blue beech (*Carpinus caroliniana*); hop hornbeam (*Ostrya virginiana*); witch-hazel (*Hamamelis virginiana*); service berry (*Amelanchier canadensis*).

REPLACEMENT IN SOUTHERN NEW ENGLAND

Three areas were selected in Connecticut as representative of southern New England:

1. The Maltby tract of the New Haven Water Co., west of New Haven. (Figs. 1 and 2, A.) This tract has been under management since 1907, and has been described by Hawley (*8, 9*).

2. The Meshomasick State Forest, north of Portland. Conditions in this forest have been described by Filley and Moss (*2*).

3. The Whittemore estate, adjacent to Lake Quassapaug, east of Woodbury—studied by Frothingham in 1910 (*3*).

Hawes (*7*) and Frothingham (*3*) have also described conditions in the second-growth forests of Connecticut before the chestnut was killed by the blight.

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[5] In Pennsylvania and New Jersey pitch pine is a class 1 species, but in New England it is very seldom associated with chestnut.

[6] Various other shrubs large enough to appear in the records were also included in this class.

STUDIES ON PERMANENT SAMPLE PLOTS

The Connecticut areas were selected for study largely because they contained permanent sample plots which were established in other studies.   Records of some of these plots are available as far
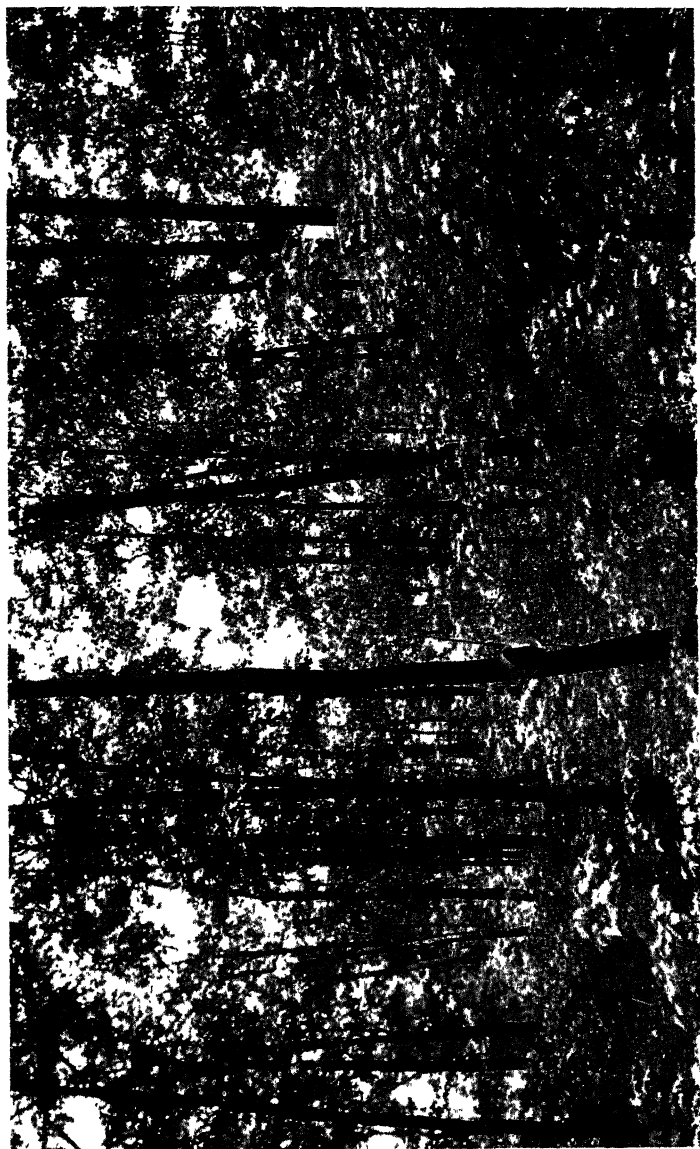
FIG. 1.—A well-managed stand of second-growth hardwoods in southern Connecticut.   The chestnut has been salvaged and the stand, now composed chiefly of oaks, has fully recovered and still contains ample advance reproduction

back as 1904, the year that the blight was first discovered in Bronx Park, N. Y.   In the present studies the plots are identified by the same numbers used in the other studies.   On these plots, each of which covered from one-tenth to one-fourth acre, all essential details,

such as species, size, and position of each tree in the forest canopy, were recorded at each periodic examination.

To determine the effect of a reproduction cutting made in 1902, plots 321 and 323 were established in 1906 in a 45-year-old stand of second-growth hardwoods on the Maltby tract. All the chestnut
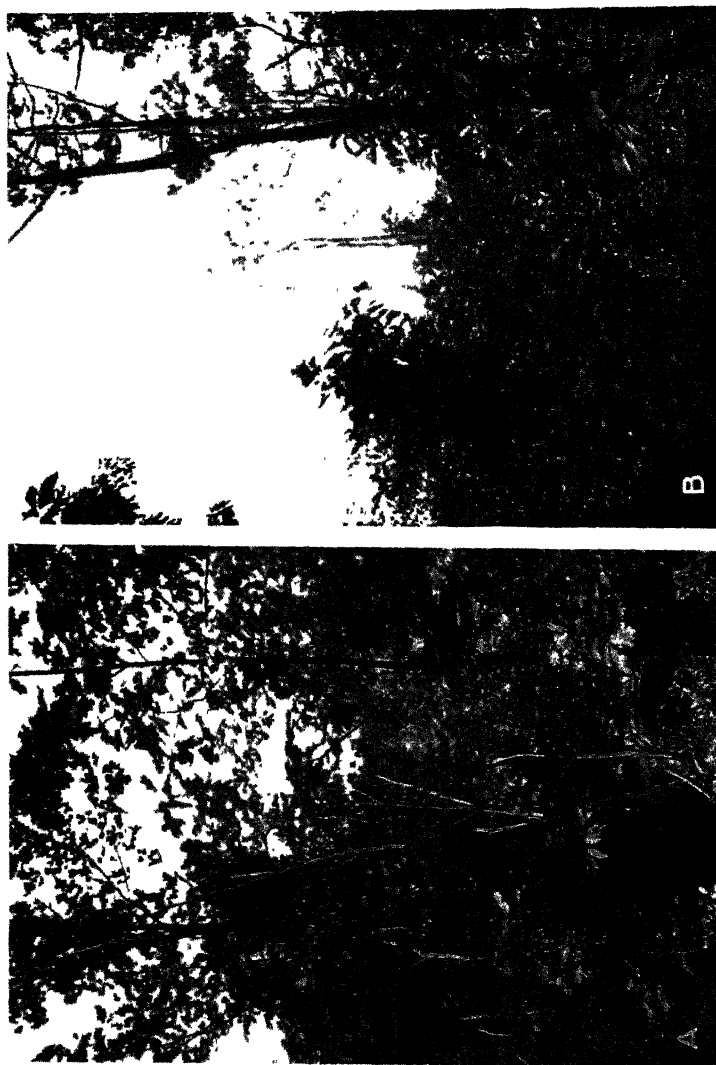
FIG. 2.—A, a young stand of hardwoods following clear cutting about 15 years before. The first three generations of chestnut sprouts have been killed by the blight and the overtopped fourth generation is heavily infected. The opening left by the chestnut has been completely filled by red oak, hickory, and yellow poplar, the crowns of which are interlaced (Maltby tract, southern Connecticut). B, a mixed stand of hardwoods and pitch pine in Sussex County, N. J. The blight-killed chestnut is being replaced by a dense growth of young hardwoods containing some young pitch pine and white pine in mixture.

on these plots was cut and salvaged in 1913. Plot 474 was laid out in 1910 in a 45-year-old stand on the Whittemore estate. A heavy thinning ("C" grade) was made the same year. In 1915 an improvement cutting was made, taking out about 1 cord per acre. In this cutting, all of the dead and suppressed trees, most of the intermediate trees, and some of the codominant trees—those crowded

from the side but with their crowns still in the upper story of the stand—were cut.   Oak was left wherever practicable because it was then evident that the chestnut would not survive the blight.   Plot 475 was located in the same stand, but received no special treatment
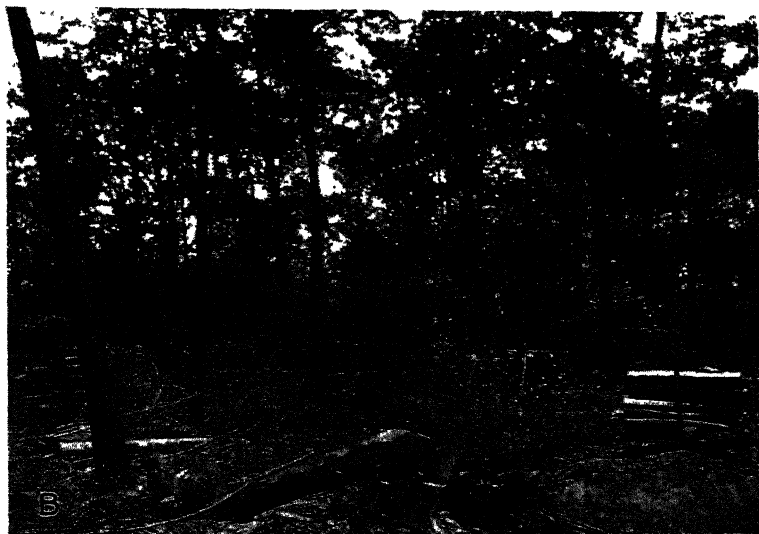
Fig. 3.—A, permanent sample plot in 1910, thinned to stimulate the growth of the stand. B, the same plot in 1924 after the blight-killed chestnut had been salvaged and the remaining stand improved by cutting out some of the less desirable individuals of other species. The material removed was used for railroad ties and charcoal

until the chestnut was salvaged in 1917–18 along with that on plot 474.
    Plot 2 was laid out in 1910 in a 45-year-old stand of second-growth hardwoods on the Meshomasick State Forest, in which very little or no cutting had been done prior to that time.   (Fig. 3.)   Plot 4 was

situated in the same locality, but in a 25-year-old stand. The first important cutting on either of these plots was made in 1924. It consisted of a chestnut salvage cutting combined with a light improvement cutting applied to the remaining stand, which ran heavily to oak. The main objects of the improvement cutting were to leave the area in the best possible condition and to favor red oak, chestnut oak, white ash, and hickory, and at the same time to keep down the proportion of red maple and other less desirable species.

At the time these plots were established the respective percentages of chestnut in the stand on the basis of basal area were as follows:
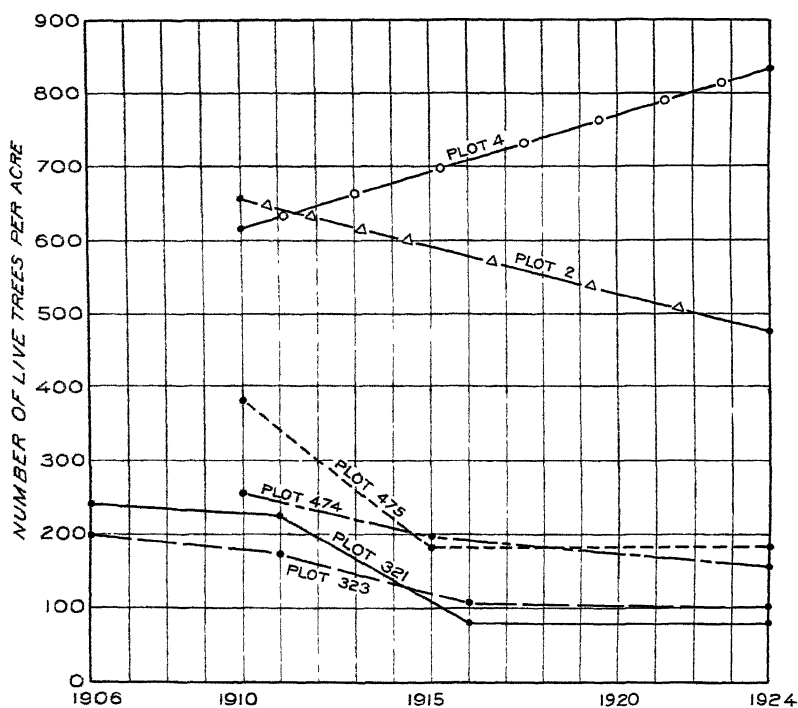
Fig. 4 —Progressive change in number of live trees per acre (1906–1924), due mainly to heavy mortality in chestnut between 1911 and 1916

Plot 321, 50; plot 323, 32.7; plot 474, 71.2; plot 475, 66.6; plot 2, 42.7; and plot 4, 44.2. At the last examination of these plots (in 1924) all essential details, such as species, size, and position of each tree in the forest canopy, were again recorded. After computing the detailed results of each successive examination it was possible to trace the history of the various changes taking place in the forest during the last 13 to 18 years. For each plot the progressive changes in total number per acre of live trees of all species 1 inch or more in diameter is shown in Figure 4. The change in basal area per acre of all live trees is shown in Figure 5.

Figure 5 shows the material decrease in basal area occurring between 1911 and 1915, when the chestnut dropped out of most of the stands. It must, however, be recalled that the improvement cuttings which

removed some material other than the chestnut also reduced the growing stock to some extent. Plots 321 and 323 have shown a slight increase in basal area since 1916 in spite of the fact that a slight decrease in number of trees occurred. It is not surprising to find stands in which the basal area is still decreasing, even though the number of trees may be increasing. This is well illustrated by plot 4, in which the death of a small number of large chestnuts more
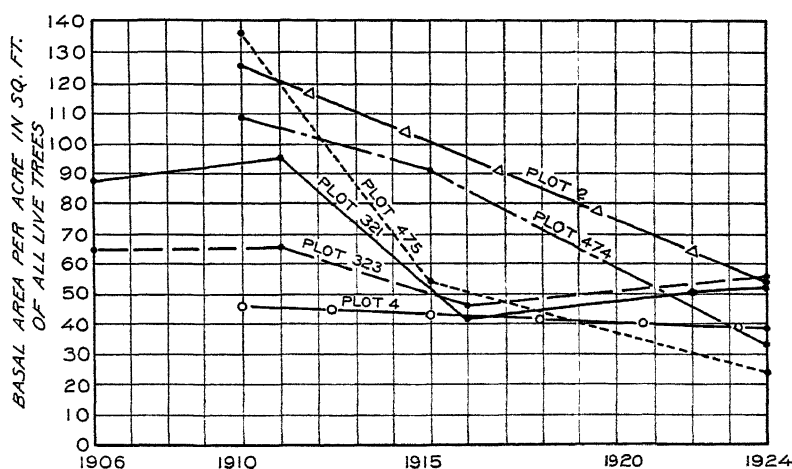
FIG. 5.—Progressive change in basal area per acre (1906–1924), due mainly to heavy mortality in chestnut between 1911 and 1916

than offset the gain in basal area of a large number of small trees which had just entered the measurable size class.

In order to generalize the results from these plots and bring out the significant changes caused by the chestnut blight, the data for all have been combined on an acre basis in Table 1, which summarizes

TABLE 1.—*Average number of live trees and average basal areas per acre for the dominant stands* [a]

Species	1910–11				1924			
	Trees per acre		Basal area		Trees per acre		Basal area	
	Number	Per cent	Square feet	Per cent	Number	Per cent	Square feet	Per cent
Chestnut	153.3	70.5	64.18	76.2	0	0	0	0
Red oak	22.0	10.1	9.13	10.8	38.0	28.6	16.45	42.2
Chestnut oak	22.0	10.1	5.25	6.2	52.7	39.7	14.48	37.2
White oak	9.4	4.4	1.97	2.3	9.4	7.1	2.07	5.3
Black oak	2.0	.9	.82	1.0	3.3	2.5	.94	2.4
Scarlet oak	8.0	3.7	2.75	3.3	5.3	4.0	3.31	8.5
Sweet birch	.7	.3	.13	.2	0	0	0	0
Red maple	0	0	0	0	8.7	6.6	.82	2.1
White ash	0	0	0	0	6.7	5.0	.32	.8
Hickory	0	0	0	0	6.0	4.5	.29	.8
Sugar maple	0	0	0	0	2.7	2.0	.26	.7
Total	217.4	100.0	84.23	100.0	132.8	100.0	38.94	100.0

[a] Includes dominant and codominant trees on permanent sample plots 321, 323, 474, 475, 2, and 4. Basal or cross-sectional area measured at 4.5 feet above ground.

the number of live trees and basal areas of the dominant stand as they were in 1910–11 and in 1924. The change in average basal area for the same six Connecticut plots is shown graphically in Figure 6. The outstanding feature of Table 1 is the increase in basal area of red oak and chestnut oak, together with an actual increase in the number of stems. The natural replacement of the original chestnut forests by stands running very largely to valuable oak is strikingly brought out by this table. While these plots were located in typical stands, some had been thinned one or more times before the 1910–11 records were taken. In 1910 the 217.4 trees per acre in the upper crown class ranged in size from 3 to 13 inches, breast high, averaging 8 inches; and now after the chestnut has died and the forest is beginning to rehabilitate itself, there are 132.8 trees per acre
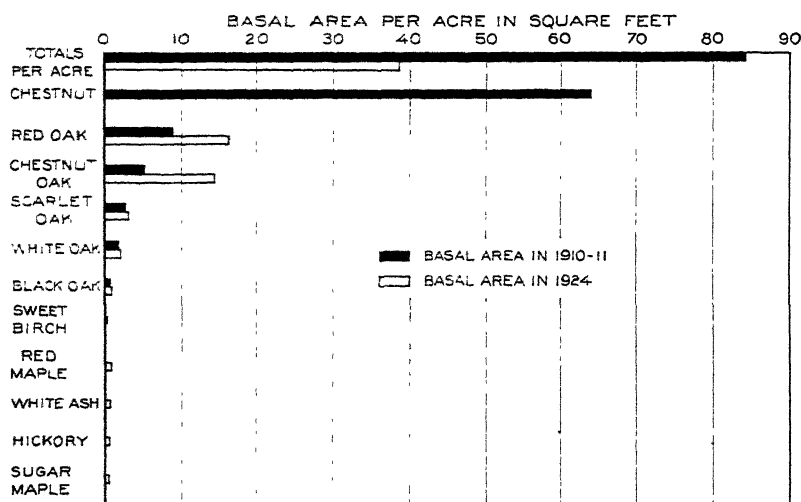
FIG. 6.—Change in basal area by species for the dominant part of the stand from 1910–11 to 1924 (graph based on averages from summary of six Connecticut plots)

in the upper crown cover, ranging from 2 to 15 inches in diameter and averaging 7.3 inches.

Permanent plots 319 and 320 are of particular interest. They are located on the Maltby tract in a stand which was clear cut about five years prior to their establishment in 1904. In 1909 chestnut comprised 24 per cent of the stand, while in 1924 the only living chestnut was sprout growth an inch or less in diameter and not more than 3 to 4 years old—overtopped, heavily infected, and dying. This area is now stocked at the rate of 785 trees to the acre in the upper crown cover, ranging from 1 to 6 inches in diameter.

The dominant stand on these plots is composed of 45 per cent class 1 species, 41 per cent class 2 species, and only 14 per cent class 3 species. Out of a total of 995 trees of class 3 species, 89 per cent are overtopped trees, which on account of their slow growth will probably not gain a position in the upper crown cover. In fact, some of these trees now in the overstory will in time be overtopped by the faster-growing species of classes 1 and 2.

A study of Figure 7, a sketch of the crown spread of the trees on permanent plot 320 in 1904, and Figure 8, a sketch of the same plot in 1924, clearly shows that the chestnut has completely disappeared from the dominant part of the stand, and that the crown cover of the dominant stand is already almost completely closed. From the standpoint of crown closure, this plot shows exceptionally good re-
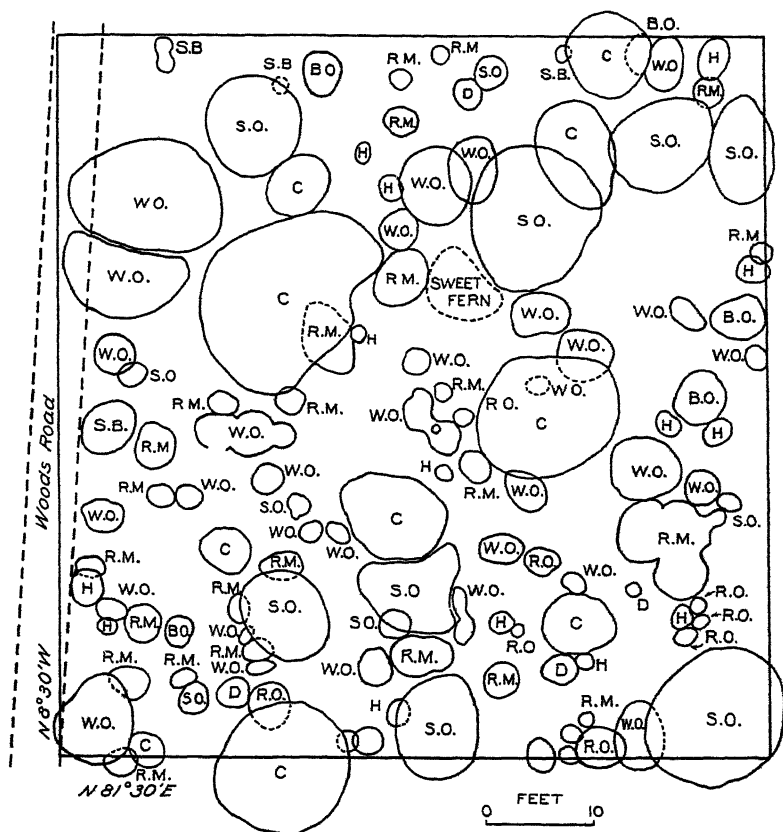
C = CHESTNUT   S.O.= SCARLET OAK   H = HICKORY
W.O.= WHITE OAK   R.O. = RED OAK   R.M.= RED MAPLE
B.O.= BLACK OAK   S B.= SWEET BIRCH   D = DOGWOOD

FIG. 7.—Crown spread of trees on permanent sample plot No. 320, as sketched in 1904 (Maltby tract)

covery. The amount of red maple in the upper crown cover of this stand is above the average for otherwise similar stands. Its preponderance is probably due to the fire which lightly burned over the area a few years after cutting. The fire favored the increase of red maple in the overstory, and dogwood in the understory. However, on sites of second quality, similar to this area, red maple is much less aggressive than it is on the better sites (*10*).

The decrease in the number of trees on most of the permanent plots is due not only to the loss of chestnut, but also to the removal

of some of the less vigorous and less desirable individuals of the oaks and other species when the chestnut was salvaged. This reduction in forest growing stock or capital is partly offset by the increased growth of the oaks. Furthermore, it must be remembered that the openings left in the forest as a result of the death of the chestnut contain a significant number of seedlings and sprouts, mostly of the valuable species as yet too small to appear in the plot records. (Fig.

W.O.=WHITE OAK   S O.= SCARLET OAK   R.M.=RED MAPLE
B.O.=BLACK OAK   H=HICKORY   D=DOGWOOD
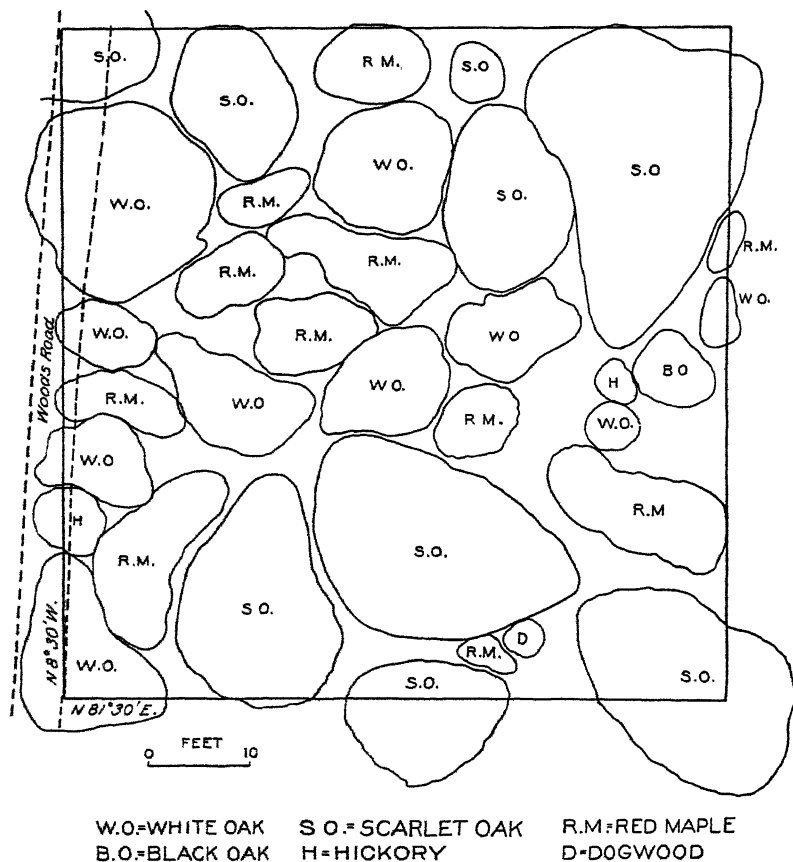
Fig. 8.—Crown spread of the stand on the same plot shown in Figure 7, as sketched in 1924. Because of the almost complete closure of the dominant stand, the overtopped trees were not mapped. The chestnut has completely disappeared from the overstory

9.) The results of a special study, aimed to determine specifically the nature and extent of restocking in the openings formerly occupied by chestnut, will be given on a subsequent page.

STUDIES ON TEMPORARY PLOTS

Records were made of the stands on 1 quarter-acre and 3 tenth-acre plots, and of the reproduction on 24 square-rod plots on the Maltby tract. The quarter-acre plot (No. 1) was laid out in a 60-year-old stand which contained about 35 per cent chestnut before it was killed by the blight. The dead chestnut poles are still standing

and have sprouted very feebly. The dominant stand contains 96 trees to the acre, of which 96 per cent are class 1 species, 4 per cent are class 2, and none are undesirable. Red oak and white oak between 5 and 14 inches in diameter form over 87 per cent of the

FIG. 9.—A blank in a stand of hardwoods left by the death of the chestnut The surrounding oaks are filling it by an extension of their crowns. The crown cover will soon be completely closed. (Maltby tract, southern Connecticut)

dominant stand. The undesirable species are practically confined to the understory. The dominant stand is reinforced by a subordinate stand of 572 trees, of which 46 per cent are class 1 species and 15 per cent are class 2. It is therefore evident that although this

stand is somewhat depleted, conditions are favorable for a satisfactory restocking.

Plots 2 and 3 were tallied in a 15-year-old stand which promptly followed clear cutting. The area had probably not been burned over since the cutting and received no subsequent treatment. Chestnut originally made up 30 per cent of the stand. The area now bears a dominant stand of 835 trees to the acre. In this stand class 1 species lead with 65 per cent, and class 2 species comprise 19 per cent; only 16 per cent of the overstory is made up of undesirable species. Here also red oak and white oak are preponderant in the overstory, with smaller quantities of hickory, beech, gray birch, sweet birch, black oak, red maple, scarlet oak, largetooth aspen, white ash, sugar maple, and basswood, in the order of their abundance. The understory contains 88 per cent of the total number of undesirable species. On the other hand it also has 1,075 trees of class 1 species, or 43 per cent of the total number, and 455, or 18 per cent, of class 2 species—more than enough to insure restocking with the better species.

Plot 4 was tallied in a stand that had been thinned quite heavily in 1906. All the chestnut was removed in 1913; the oaks were left until 1921, when they, too, were cut. The resulting stand, on an acre basis, has a total of 2,590 saplings, at present receiving practically full light from above. Of these, 32 per cent are class 1 species, 54 per cent class 2 species, and only 14 per cent class 3 species. Since red maple constitutes 34 per cent and dogwood 9 per cent of the overstory, cleanings or thinnings may be advisable to increase the proportion of the more desirable species in this stand. A similar operation in a permanent sample plot is shown in Figure 3. Scarlet oak, red oak, hickory, white oak, sweet birch, sugar maple, and beech follow in the order of relative abundance. These vary from 330 scarlet oak saplings to 110 beech. Of the class 1 species 72 per cent are in the overstory. Of the undesirable species 53 per cent are already overtopped. In the overstory 60 per cent of the class 1 species, 17 per cent of the class 2, and 19 per cent of the class 3 species are of seedling and the remainder of sprout origin. The combination of desirable species and desirable (seedling) origin is particularly noteworthy.

All the plots considered thus far fail to bring out clearly the nature and extent of replacement within the gaps left by the chestnut and the proportion of shrub or inferior species which may be taking the place of the valuable chestnut. To obtain this information 24 square-rod sample plots were laid out and tallied in Connecticut in 1924, and records were made of the forest-tree reproduction and of the larger or more abundant species of shrubs above 6 inches in height. Each plot was laid out so that the center of the clump of dead chestnut formed the center of the plot. Plots were tallied in four representative stands, varying from recently clear-cut stands (plot 4 above) to a 60-year-old stand in which the merchantable chestnut had not yet been salvaged (plot 1 above). The records of these plots are summarized in Table 2. Attention should be directed to the fact, not brought out in the table, that the live chestnut sprouts seldom attain a diameter of more than an inch. The third and fourth generations of sprouts were being killed by the blight at the time the tallies were made. Two or three sprout generations may still be expected on the

clear-cut areas and on those on which the chestnut was salvaged before it died. Many of the clumps of chestnut which were not cut before the trees died have already ceased to sprout.

TABLE 2.—*Reproduction under blight-killed chestnut, southern New England* [a]

Plots			Chestnut reproduction per square rod			Other reproduction per square rod [b]					Percentage of area on which tallest saplings are found	
							Class 1 species		Class 2 species			
Nature	Number	Average diameter of chestnut clumps	Dead sprouts	Live sprouts	Live seedlings	All species	Sprouts and seedlings	Proportion of seedlings	Sprouts and seedlings	Proportion of seedlings	Class 1 species	Class 2 species
		Feet	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Stand clear-cut in 1921 (plot 4)_	8	11.4	17.1	11.0	0.6	44.6	32	55	24	11	60	27
Stand clear-cut in 1908 (plots 2 and 3)_____	6	8.0	17.0	7.9	.0	24.7	60	74	18	15	79	0
Permanent plot 474 [c]_____	5			28.0	1.6	93.8	75	59	11	45		
60-year-old stand (plot 1)_____	5	19.3	16.0	10.6	0	43.4	41	69	3	57	67	33
Weighted averages_____		12.4	16.8	13.7	.5	49.6	49.8	63.5	15.4	28.7	67.8	20.1

[a] Summaries of tallies of 24 square-rod reproduction plots, grouped by stands of essentially similar age or treatment; Maltby tract, near New Haven, and Whittemore estate, near Woodbury, Conn.
[b] Class 1 species are: White oak, red oak, black oak, chestnut oak, white ash, hickory, sweet birch, black cherry, basswood, sugar maple, and yellow poplar. Class 2 species are: Scarlet oak, red maple, beech, and aspen. Class 3 species are included only in the total number of all species.
[c] For description see preceding section of text.

The most striking point brought out by Table 2, aside from the fact that the blanks are now filled by a much larger number of seedlings and sprouts than can possibly survive, is that practically half of the new reproduction is of desirable species and that about 65 per cent of these are species which at present have an economic value. Furthermore, on over two-thirds of the area represented by the plots the tallest saplings are class 1 species, and on another 20 per cent they are class 2 species. Over 63 per cent of the class 1 reproduction is of seedling origin.

The goodly portion of red oak in the replacement reproduction on so many of the plots is especially noteworthy. The importance of red oak, because of its rapid growth in height and diameter and the excellent character of its timber, has long been recognized by foresters (*3, 6*). Spaeth (*15*) has classed both red oak and white ash as important species in replacing chestnut. There is little doubt that these species will increase rapidly in importance now that their formidable rival—the chestnut—has been destroyed. The second-growth hardwood type will be easy to maintain and will form the basis of future forest management on the better soils.

The most significant facts brought out by the 1924 Connecticut studies, as well as by those made in 1920, are that there is generally sufficient advance growth of valuable species to restock the stands, and that the blight-killed chestnut has been very largely replaced by

oak. (Fig. 1.) On areas originally occupied by chestnut, oak reproduction was found very generally, together with reproduction of such other valuable species as hickory, white ash, sugar maple, and sweet birch. (Fig. 2, A.)

These studies, made under representative conditions, indicate that the blight-killed chestnut stands of southern New England are restocking naturally to silviculturally desirable and economically valuable species. These species are much in excess of the undesirable shrub-weed species. If, as is occasionally true, the more desirable species are outnumbered by the undesirable ones, they nevertheless, having the more rapid growth rate, will soon overtop the inferior species and partially or completely suppress them. It is also encouraging to note that in those cases where the total number of desirable species may not equal that of the undesirable ones, the desirable species in the overstory are already in excess of the others. Moreover, the tallest saplings in the gaps left by the death of the chestnut are mostly of desirable species, such as the oaks, hickory, and ash, rather than of shrub or weed species, such as dogwood, sassafras, blue beech, and gray birch.

REPLACEMENT IN NEW JERSEY

Since no permanent sample plots were available for study in New Jersey, it was necessary to resort wholly to temporary plots. Three quarter-acre plots were laid out in uneven-aged stands on the Stokes State Forest, in Sussex County, northwestern New Jersey. Chestnut, at the time of its death, comprised 30 to 60 per cent of the stand. In this locality the chestnut had been dead a shorter time (mostly less than five years) than in the regions to the south and east. The first generation of sprouts had died, and the second generation was appearing.

Plot 1 is located on a moist flat in what was originally an oak-chestnut-pine forest. The overstory of the stand contains 1,316 trees to the acre, of which 12 per cent are class 1 species, 40 per cent class 2 species, and 48 per cent class 3 species. The class 1 species, in the order of their abundance, are white oak, chestnut oak, and white pine, while the class 2 species, in the order of their importance, are red maple, scarlet oak, black gum, and largetooth aspen. In addition to these, the overtopped stand contains about 600 trees to the acre, some of which are capable of increased growth when released, or of filling the gaps left by the chestnut.

Plots 2 and 3 are located on a low ridge in an uneven-aged stand which originally was an oak-chestnut forest. These plots have an average stand of 1,879 trees to the acre in the overstory, of which 34 per cent are class 1 species, 18 per cent are class 2 species, and 48 per cent are class 3 species. Of the desirable species over 60 per cent are in the upper crown class. These stands are obviously in need of an improvement cutting aimed especially at increasing the proportion of desirable species, which was reduced about 20 years ago by a cutting that culled out most of the best pitch pine. Reproduction of desirable species is rather deficient because of the heavy cover of mountain laurel (*Kalmia latifolia*) and scrub oak. These undesirable species will in time be shaded out by the tree species, the scrub oak being the first to give way. With protection from fire the scrub oak is very short lived, for on account of its intolerance of

shade it can not compete with any tree growth which overtops it *(1)*. If the stand is allowed to develop without artificial treatment, it will eventually become an almost pure stand of the better species of oak, with some maple and a small quantity of pitch pine. It will, however, be more irregular, less well stocked with the better species, and slower in growth rate than with treatment. Although the pine is here somewhat more aggressive as a replacement species, particularly in filling larger openings left by the death of the chestnut, many of the pine seedlings are lacking in vigor and will probably be overtopped by the hardwoods.

Fifteen reproduction plots similar to the 24 small plots in the New England study were also laid out, the center of the plot coinciding with the center of the chestnut clump. The results obtained on these plots are summarized in Table 3. It is evident that there is an ample supply of forest-tree reproduction to fill the gaps, although the percentage of class 1 species is much lower than was found in southern New England. With less than half of the reproduction of species other than chestnut falling in classes 1 and 2, the desirability of modifying the composition of the stand is emphasized. It is encouraging, however, to note that the tallest saplings on slightly over 80 per cent of the area represented by the plots are either class 1 or class 2 species, although the percentage of class 1 is still small.

The stands studied in New Jersey were characterized by the presence of white pine and pitch pine, and by a higher percentage of the undesirable species in the overstory and a denser understory and undergrowth than those studied in Connecticut. (Fig. 2, B.) Although the chestnut is being replaced naturally for the most part by tree species, yet the presence of a relatively high proportion of undesirable species introduces important problems in connection with subsequent thinnings and improvement cuttings.

TABLE 3.—*Reproduction under blight-killed chestnut, New Jersey* [a]

Plots			Chestnut reproduction per square rod				Other reproduction per square rod [b]					Percentage of area on which tallest saplings are found
							Class 1 species		Class 2 species			
Nature	Number	Average diameter chestnut clumps	Dead sprouts	Live sprouts	Live seedlings	All species	Sprouts and seedlings	Proportion of seedlings	Sprouts and seedlings	Proportion of seedlings	Class 1 species	Class 2 species
Uneven aged; oak-chestnut forest; flat ridge (plots 2 and 3)_____	10	*Feet* 20. 4	*Number* 18. 8	*Number* 20. 8	*Number* 2 1	*Number* 55. 2	*Per cent* 23	*Per cent* 96	*Per cent* 22	*Per cent* 84	*Per cent* 20	*Per cent* 56
Uneven aged; oak-chestnut-pine forest, flat (plot 1)_____	5	18. 5	13. 6	22. 4	. 6	45. 8	13	61	34	75	27	64
Weighted averages_____		19. 8	17. 1	21. 3	1. 6	52 1	19. 7	84 3	26	81	22. 3	58. 7

[a] Summaries of 15 square-rod plots tallied on the Stokes State Forest in northwestern New Jersey.
[b] Species in the order of their abundance are: Class 1, red oak, black oak, chestnut oak. white oak, hickory, sweet birch, white pine, and pitch pine; class 2, scarlet oak, red maple, black gum, and aspen; class 3 species are included only in the total number of all species.

An earlier sample-plot field study, made by Richards *(14)* in Somerset County, N. J., is of interest with respect to both his results and conclusions, the conclusions being at variance with those of the present studies. Chestnut oak was found to be the predominant species in the reproduction, amounting to 44 per cent on the ridge tops and 22 per cent on the slopes. On the ridge tops 11.8 per cent was hickory, 6.8 per cent red oak, and 3 per cent white ash. These four were the most valuable species, and comprised 65.6 per cent of the reproduction on the ridge tops. The remainder was made up of red maple, cherry, butternut *(Juglans cinerea)*, birch, and sassafras. Reproduction on the slopes, in addition to chestnut oak, was made up of 6 per cent red oak and 10 per cent pignut hickory *(Hicoria glabra)*, besides some cherry, maple, and butternut.

Because the desirable species, other than chestnut oak, were in the minority, Richards concluded that there was little reproduction in the way of desirable native species with which to form the new forest, and consequently recommended replacement of the chestnut by planting Norway pine *(Pinus resinosa)*. However, Richards's study was mostly of areas cut over less than a year previously (a few of his plots had been cut over seven years), so that in all probability most of the reproduction he found was advance growth and much of the seedling growth prominent in the replacement had not yet become established. Had his study been made a few years later, on areas cut over for a longer time, he would probably have found more reproduction and a higher proportion of desirable species, and accordingly less occasion for the planting of pine.

REPLACEMENT IN PENNSYLVANIA

The studies of natural replacement were not so intensive in Pennsylvania as in Connecticut and New Jersey, since this problem was already being studied by J. S. Illick and assistants, for the Pennsylvania Department of Forests and Waters.

Two sixteenth-acre plots laid out near Lehigh Gap, Pa., in 1920, are of special interest. One plot was on an area which, at the time it was clear-cut three years before, contained 62 per cent chestnut. The new stand contained 9,424 live trees to the acre, of which 22 per cent were chestnut sprouts. Of the remaining live trees, 86 per cent were class 1 species, chestnut oak, white oak, and sweet birch; 14 per cent were class 2 species, scarlet oak, red maple, black gum, and cottonwood; and none were undesirable species. The other plot was located in a stand cut heavily for chestnut 25 years earlier. This stand originally included 93 per cent chestnut, but in 1920 the proportion of chestnut sprouts in the live stand had been reduced to 32 per cent, and by 1924 the chestnut had been entirely eliminated. The stand contained a total of 10,234 trees to the acre, exclusive of chestnut, of which 40 per cent were class 1 species, white oak, black oak, sweet birch, red oak, black cherry, and hickory; 55 per cent were class 2 species, red maple, scarlet oak, black gum, and cottonwood; and only 5 per cent were undesirable. These stands have obviously restocked mostly to desirable species, and almost as rapidly as the chestnut disappeared from the stand.

Stand records, obtained in company with T. E. Shaw, of the State Forest School in the Mont Alto Forest in Franklin County, show that on slopes where the chestnut had occupied 40 per cent or more of the original stand seedlings and sprouts of other species varied from 1,000 to 1,500 per acre. Of these, chestnut oak comprised 60 per cent, black oak 35 per cent, and white oak 4 per cent. Scattered red and scarlet oaks, white and pitch pines, and black locusts made up the remainder. Here the oaks, which in the original stand were second in number to the chestnut, have insured adequately stocked stands of second growth. Chestnut oak, pitch pine, and black locust (*Robinia pseudoacacia*) are also among the desirable species which are largely replacing the chestnut in Pennsylvania. Black oak, pignut hickory, sweet birch, mountain pine (*Pinus pungens*), and Virginia pine (*P. virginiana*) are others which are helping to fill the gaps, and are not to be despised (*11*).

The Pennsylvania studies also showed that the blighted areas are being restocked by natural reproduction, mostly of desirable species. The replacement is more complete and more satisfactory in moist situations than upon the drier slopes, for blanks on the moist sites are relatively less extensive and the associated species, such as yellow poplar, white ash, red oak, white pine, red maple, and white oak, more desirable. Upon the drier slopes the blanks are more extensive, and the associated species are less desirable.

SUMMARY

This paper presents the results of studies to determine the character and amount of natural replacement of blight-killed chestnut. They were begun in the earlier infected chestnut stands of the Northeast, where natural replacement is most advanced and could most easily be studied.

In southern Connecticut permanent sample plots established between 1904 and 1910, as well as temporary plots, were studied. In New Jersey it was necessary to resort wholly to temporary plots. Limited studies were also made in Pennsylvania.

The natural replacement of the forests, originally containing large quantities of chestnut, by stands running very largely to oak—red oak, white oak, chestnut oak, black oak, or scarlet oak—is strikingly brought out by these studies. An outstanding feature is the increase of red oak and chestnut oak both in number of trees and basal area. Such other desirable species as hickory, white ash, sugar maple, and sweet birch may be associated with the oak.

While the stands are for the most part still depleted in basal area they are recovering satisfactorily. From the standpoint of crown closure the areas studied also showed exceptionally good recovery. The stand remaining after the death of the chestnut, composed chiefly of oaks, has responded to increased light, and the crowns of these trees are very effectively closing the smaller openings left by the chestnut.

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ISOLATION AND IDENTIFICATION OF SOME ORGANIC NITROGENOUS COMPOUNDS OCCURRING IN ETIOLATED CORN SEEDLINGS [1]

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INTRODUCTION

There are in the literature many records concerning the occurrence of asparagine in plants. These records refer chiefly to legumes, although other plants, too, were found to contain that amino acid. Thus, as early as 1867, Beyer (*1*)[2] found asparagine in seedlings of the yellow lupine while von Gorup-Besanez (*3, 4*) reported its occurrence in vetch seedlings. The finding of Beyer was corroborated by Schulze (*14*), by Schulze, and Umlauft (*21*), as well as by Schulze and Barbieri (*17*), while the report of von Gorup-Besanez was confirmed by Schulze, Steiger, and Bosshard (*22*). From the extensive work of Schulze and his collaborators we know also that asparagine is found in potato tubers (*16*), young lucern plants (*15*), oat plants (*22*), etiolated soy-bean seedlings (*13*), asparagus, celery, dahlia bulbs (*15*), and other plants (*18*). Frankfurt has also reported the occurrence of asparagine in the wheat embryo (*2*). Of the cereals only the wheat embryo and young oat plants have been reported to contain asparagine, as far as the writer is aware. After it had been shown in a previous paper (*9*) that a great part of the proteins of the maize kernel is converted into amino acids, polypeptides, and other degradation products by the process of germination, it was of interest to find out the exact nature of the individual cleavage products, since the latter differ from each other in many respects from the standpoint of nutrition and physiology. It was to acquire this additional information that the present investigation was undertaken. In it the occurrence of asparagine and vernin in etiolated corn seedlings is reported.

METHODS AND RESULTS

PRELIMINARY ANALYSES OF SEED

Prior to starting germination experiments it was thought advisable to secure some data regarding the composition of the seed. Accordingly, several determinations were made, with the results recorded in Table 1.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No 7
Apr. 1, 1927.
Key No. G-574

(649)

TABLE 1.—*Proportion of moisture and ash, and of total nitrogen, protein nitrogen, and nonprotein nitrogen in the ungerminated corn kernel*

Variety	Moisture in—		Ash in—	Total nitrogen in—	Protein nitrogen in—		Nonprotein nitrogen in—	
	Air-dry kernels	Air-dry flour	Oven-dried kernels	Oven-dried flour	Oven-dried flour	Total nitrogen	Oven-dried flour	Total nitrogen
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Iodent (1924 crop)____	11.48	9.15	1.57	1.67	1.56	92.81	0.18	10.57
Do_____	11.27	9.35	1.39	1.69	1.53	90.97	.17	9.92
Do_____			1.48	1.67	1.57	93.20	.14	8.53
Average_____	11.38	9.25	1.48	1.68	1.55	92.33	.16	9.67

In connection with Table 1 it may be mentioned that the flour represents the whole maize kernel, which was ground finely enough to pass a 40-mesh sieve. A moisture determination of the kernel was made in order to have a basis for the calculation of the results obtained on the oven-dried kernel. The protein nitrogen was determined by the Stutzer method, referred to in previous papers (*8, 11*).

METHODS OF GERMINATION

The seed (Iodent variety) was spread on perforated metal trays, ordinarily from 350 to 400 kernels per tray, between moist absorbent paper towels, and allowed to remain in a dark germinator at a temperature of from 25° to 27° C. The seedlings were usually watered once a day with distilled water. At the expiration of five to seven days the sprouts were severed from the kernels and dried in an electrically-heated oven at 55°–60° for about two days, and then ground and passed through a 40-mesh sieve.

EXTRACTION

Ordinarily, 100 gm. portions of the flour were treated with boiling ammonia-free water, shaken well and heated on the steam bath for at least 15 minutes. The whole was now centrifuged or filtered on a Büchner funnel with suction. The solid residues were extracted once more in like manner. To the combined extracts subacetate of lead was added as long as a precipitate came down. A slight excess of the precipitant was used. The precipitate was allowed to stand 24 hours, at the expiration of which it was filtered off with suction and washed with water.

ISOLATION

Filtrate and washings were now treated with a solution of mercuric nitrate. After this treatment the whole was made neutral to litmus with sodium hydroxide solution and allowed to stand for 24 hours. When the precipitation was complete the precipitate was collected on a filter and washed with cold water. The washed precipitate was then suspended in water and decomposed with hydrogen sulphide. The mercuric sulphide precipitate was filtered off and the clear filtrate neutralized carefully with a few drops of strong ammonia and evaporated on the water bath at low temperature to a thin sirup a drop or two of ammonia being added from time to time to keep the reaction

neutral.   When sufficiently concentrated the substance was allowed to solidify slowly in a vacuum over sulphuric acid.   It was noticed that with increasing concentration the sirup gradually assumed a dark, almost black color, being so sticky that it could not be removed from the crystallizing dish.   The sirup was readily soluble in water and practically insoluble in alcohol.   When alcohol was added to the aqueous solution a dark brown sediment gradually formed at the bottom of the vessel, the supernatant liquid becoming lighter in color.   When spread on porous, unglazed porcelain the sirup gradually became free from the sticky substance.   The whole of the sirup was purified by taking advantage of the two observations made.

After the solid substance had been removed from the unglazed porcelain it was dissolved in hot water, filtered, and allowed to concentrate and crystallize in vacuo over sulphuric acid.   The almost dry, impure, crystallized mass obtained weighed 16 gm.   It was procured from 1,200 gm. of air-dry corn sprouts whose ungerminated kernels had the composition shown in Table 1.   After several recrystallizations from hot water the fair-sized crystals, which could be picked out without difficulty, became clear and practically colorless. They had the form of rhombic prisms and will be referred to in this paper as "Crystals A."   On removal of these crystals the remaining substance was dissolved in hot water, filtered, and again allowed to crystallize in a vacuum over sulphuric acid.   This mother liquor was expected to give another crop of "Crystals A."   However, the crystals now obtained differed from "Crystals A" in their solubility and other properties, having the form of long, fine needles.   They will be referred to as "Crystals B."   The filtrate from these crystals gave another crop of "Crystals A," which, like the first crop, was purified by recrystallization from hot water.

IDENTIFICATION OF "CRYSTALS A"

A macroscopic and microscopic examination of the crystals showed them to have the form of rhombic prisms.   They were hard, lustrous, translucent, and of considerable size when permitted to crystallize slowly.   Some of them were about 7 mm. long.   The crystals were easily soluble in hot water, difficultly soluble in cold water, and insoluble in alcohol and ether.

CHEMICAL REACTIONS

A few crystals were dissolved in water and the following tests made:
(1) A portion of the solution was heated with dilute sodium hydroxide.   When wet red litmus paper was exposed to the fumes it turned dark blue, showing the evolution of ammonia.
(2) Another portion of the solution, heated with dilute hydrochloric or sulphuric acid, neutralized with sodium hydroxide, and tested with Nessler's reagent, gave at once a reddish-yellow precipitate, characteristic of ammonia.
(3) Neutralized formaldehyde added to the neutral aqueous solution of the substance produced an acid reaction.
(4) An acid solution of the substance was dextrorotatory, while an alkaline solution was levorotatory.
(5) When treated with Millon's reagent the solution remained perfe ,tly clear and colorless, showing that the substance was free from tyrosine.

The evolution of ammonia, when a solution of the isolated substance was heated with sodium hydroxide (reaction No. 1), may have been caused by the presence in it of an acid amide or an amino acid group. To decide this question definitely, reaction No. 2 was made. Since amino acids are resistant to the action of dilute acids, reaction No. 2 plainly shows that the isolated substance has an acid amide group. On the other hand, reaction No. 3 shows conclusively that the substance has also an amino acid group. The further fact that the substance is optically active (reaction No. 4) indicates that the amino group is very likely in the α-position, since all α-amino acids, with the exception of glycin, have an asymmetric carbon atom to which the optical activity is due. Inasmuch as mercuric nitrate is known to precipitate chiefly asparagine, glutamine, allantoin, nucleic bases, and tyrosine, and inasmuch as only asparagine and glutamine have both the acid amide and the amino acid group, the idea suggested itself that "Crystals A" might be asparagine or glutamine. This question was answered by the several quantative determinations given below.

WATER OF CRYSTALLIZATION

Two portions, 0.1585 and 0.2745 gm., respectively, dried at 103° C. to constant weight, lost 0.019 and 0.033 gm., corresponding to 11.99 and 12.02 per cent of moisture. Asparagine, $C_4H_8N_2O_3 + H_2O$, requires 11.99 per cent of water.

SPECIFIC ROTATION

The specific rotation was determined with a half-shade polarimeter having a sugar scale, and the readings recalculated to degrees of circular polarization by the use of the factor 0.346. White light filtered through a dichromate cell was used. The following specific rotation tests were made:

(1) A quantity of "Crystals A" weighing 0.7505 gm. was dissolved in 25 c. c. of N/5 NaOH and made up with water to 50 c. c. Thus, a solution was obtained each cubic centimeter of which contained 0.01501 gm. of the crystals. This solution, observed in a 2 dcm. tube, gave α = observed rotation $-0.8°$ C.

(2) To 10 c. c. of the solution (= 0.1321 gm. of oven-dried crystals) 5 c. c. of N/5 HCl were added to neutralize the solution, after which 4.6 c. c. more of concentrated hydrochloric acid were added, a 10 per cent hydrochloric acid solution resulting. This solution, observed in a 2 dcm. tube, gave α = $+1.5°$. Hence, $[\alpha]_D$ = $+38.50°$.

(3) As a third test 0.2535 gm. of crystals (= 0.2231 gm. of oven-dried substance) was dissolved in a 10 per cent hydrochloric acid solution and made up with the acid to 15 c. c. The observation, in a 2 dcm. tube, gave α = $+3.1°$. Hence $[\alpha]_D$ = $+36.07°$. According to Schmidt (*12, p. 528*), $[\alpha]_D$ of asparagine in a 10 per cent hydrochloric acid solution is equal to $+37.45°$.

NITROGEN DETERMINATION

Two portions of 0.1 gm. each of the isolated crystals were oxidized according to Kjeldahl's method. They gave 18.490 and 18.570 gm. corresponding to 18.49 and 18.57 per cent, respectively, of nitrogen. Asparagine, $C_4H_8N_2O_3 + H_2O$, requires 18.67 per cent of nitrogen.

For the principles underlying the formol titration method the reader is referred to the papers of Sörensen and his collaborators (*5, 6, 23, 24*), as well as those of the writer (*7, 10*). A quantity of "Crystals A," weighing 0.7505 gm., corresponding to 5 millimoles of asparagine, was dissolved in 25 c. c. of N/5 NaOH and made up with water to 50 c. c. Thus, a N/10 solution was obtained each cubic centimeter of which had received 0.5 c. c. of N/5 NaOH in advance of titration. Two 10 c. c. portions of this solution formol-titrated, with phenolphthalein as indicator, each required for their neutralization 0.03 c. c. of N/5 HCl, corresponding to 99.4 per cent of asparagine. Under similar conditions Sörensen (*23, p. 79*) found 99 per cent of asparagine. From 1,200 gm. of air-dry corn sprouts (5 to 7 days old) there were obtained about 12 gms. of pure asparagine, or 1 per cent of the total air-dry weight of the sprouts.

To sum up, the form of crystals and the chemical reactions, as well as the specific rotation, indicate the substance to be asparagine. Furthermore, the specific rotation and the formol titration, as well as the nitrogen and moisture estimation of "Crystals A," establish beyond any doubt that the isolated crystals are asparagine.

The considerable quantity of asparagine found in etiolated corn seedlings is noteworthy. After the proteins of the corn kernel have been decomposed to amino acids by the process of germination (*9*), the latter are gradually converted into asparagine, in accordance with Schulze's theory. As an amino acid, asparagine has direct nutritive value, but its physiological importance as a means of translocating nitrogen is of greater interest.

PARTIAL IDENTIFICATION OF "CRYSTALS B"

While the full identification of "Crystals B" will require considerable time and labor, a few preliminary data may not be out of place here. On concentration in a vacuum the filtrate from the first crop of asparagine gave crystals which, on being filtered off with suction, consisted of a light voluminous, brownish-yellow mass. The mother liquor from these crystals gave, on concentration, a second crop of asparagine whose mother liquor yielded a small portion of "Crystals B." Altogether about 1.2 gm. of these crystals was obtained.

PROPERTIES OF THE CRYSTALS

The crystals (perhaps not perfectly chemically pure) are readily soluble in cold alcohol and ether, but insoluble in cold water, and difficultly soluble in hot water. The substance usually crystallizes from these solvents in long, fine, yellowish, microscopic needles, often arranged in raylike bunches. When allowed to crystallize slowly from hot water, it appears in fine, very long needles, some of which are more than 1.5 cm. in length. For purification the crystals were dissolved in absolute alcohol, filtered,[3] and concentrated on the water bath at low temperature. Long, light, silky, orange-yellow needles were formed. They have a slightly bitter taste and melt at about 142° C. to a brown-red liquid which solidifies at about 128°,

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[3] The small residue on the filter contained asparagine.

and melt again at 142°. The solution of the crystals gives a strong positive reaction with Millon's reagent, indicating the presence of a free phenol group. The solution gives a negative reaction with Mörner's reagent, showing that the crystals were free from tyrosine. This substance was not further identified at this time.

IDENTIFICATION OF VERNIN

When the alcoholic solution of the second crop of "Crystals B" (see p. 653) was filtered off through a paper filter, there remained on the paper about 0.74 gm. of a substance which differed from both asparagine and "Crystals B." It had the form of an amorphous brownish-gray powder which was insoluble in alcohol and ether but readily soluble in water, especially in hot water. It was also soluble in dilute hydrochloric and nitric acids, as well as in dilute ammonia.

CRYSTALLIZABILITY OF THE SUBSTANCE

The brownish-gray powder was dissolved in hot water, filtered, concentrated on the water bath, and placed in a vacuum over sulphuric acid. It separated practically in the amorphous state. When the amorphous substance was treated again in the same manner, it was noticed that it gradually assumed the crystalline form. The impure substance showed under the microscope the presence of a few asparagine crystals. When it had been treated repeatedly with hot water, as outlined above, the substance was finally obtained as a crystalline mass. In the attempts to obtain the substance in the crystallized state it was observed that when the almost saturated solution of the substance was placed in a desiccator the whole of it solidified to a crystallized mass within a few minutes. To the naked eye the crystals appeared mostly as long, fine needles, while under the microscope long, water-clear, sometimes very narrow and thin prisms, imbedded in a brownish liquor, could be observed. The crystallized mass was mixed well with 95 per cent alcohol, filtered off with suction, and washed with alcohol.

CHEMICAL REACTIONS

The aqueous solution of the substance was neutral to litmus paper. A solution of the substance in dilute hydrochloric acid, treated with phosphotungstic acid, gave immediately a yellowish-white precipitate. With silver nitrate the aqueous solution of the substance gave a characteristic white precipitate, soluble in ammonia. When some of the substance was dissolved in dilute nitric acid and evaporated on the water bath there remained a yellowish residue which, on being wetted with ammonia, assumed an intensified yellow color with a reddish tint. Judging from its description as given by Schulze and his collaborators (*19, 20, 22*), its solubility and crystallizability, the form of its crystals, and its reactions, the amorphous body is vernin. The workers mentioned reported this substance in young plants of vetch (*Vicia sativa*), clover (*Trifolium pratense*), pumpkin (*Cucurbita pepo*), ergot-infected rye (*Secale cornutum*) (*19*), and in the pollen grains of *Corylus avellana* and *Pinus silvestris* (*20, 22*).

To further identify the amorphous body the crystalline material was thoroughly mixed with 95 per cent alcohol, filtered, and washed with alcohol. It was then dried in the air for 36 hours.

Quantities weighing 0.3800 and 0.1601 gm. of air-dry substance lost 0.0441 and 0.0184 gm. at 105° C. corresponding to 11.60 and 11.49 per cent, respectively, of water. The formula for vernin, $C_{16}H_{20}N_8O_8 + 3H_2O$, requires 10.68 per cent of water.

Quantities of oven-dried substance weighing 0.1410 and 0.1521 gm. oxidized according to the Kjeldahl method gave 0.0336 and 0.0361 gm., corresponding to 23.83 and 23.73 per cent, respectively, of nitrogen. The formula, $C_{16}H_{20}N_8O_8$, requires 24.78 per cent of nitrogen.

The analyses given suggest that the isolated vernin was not entirely pure. Unfortunately, a part of the substance, including a preparation of the silver compound, was lost by accident. However, as the preparation of more substance will require much time, it is deemed best to publish, in the meantime, the results obtained in this study by which the identity of vernin seems reasonably well established. Inasmuch as Schulze (*15*) has shown that vernin yields, on hydrolysis, a pentose and guanin, a hydrolytic product of nucleic acid found in the cell nucleus, it seems reasonable to assume that vernin may play a rôle in the synthesis of nucleoproteins.

SUMMARY

Asparagine has been shown to occur in etiolated corn seedlings. It was isolated and identified by its chemical reactions, by its specific rotatory power and its formol titration, as well as by its water and nitrogen estimation.

The occurrence of vernin in etiolated corn seedlings seems to be indicated.

A few preliminary data are given on what appears to be an orange-yellow pigment met with in etiolated corn seedlings.

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THE EFFECT OF APPLICATIONS OF CYANAMID ON THE NITRATE CONTENT OF FIELD SOILS [1]

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INTRODUCTION

Availability studies [2] with various nitrogenous fertilizers for cotton, conducted by the Fixed Nitrogen Research Laboratory, have indicated that in cases where cyanamid failed to give as good results as other carriers of nitrogen, one of the chief causes was the failure of the material to nitrify readily. Laboratory and greenhouse experiments [3, 4, 5] have contributed additional data along the same lines and have shown further that some of the products formed from cyanamid in the soil, particularly dicyanodiamide, are very toxic to the nitrifying bacteria. Experiments of numerous other investigators, discussed in the publications mentioned, have shown similar indications. In order to secure data on the rate of nitrate formation that might be more closely coordinated with the behavior of plants growing in the field, a series of nitrification studies was started in which soils from three of the experimental fields at Muscle Shoals, described in one of the publications cited [2] were used. These experiments were conducted at the same time that the cotton experiments were in progress and give a good idea of the nitrate content of the soils while the crop was growing.

PROCEDURE

The method of experimentation adopted was the usual procedure for nitrification experiments. The soils from the experimental fields were air-dried and 100 gm. samples weighed out into 200 c. c. beakers. To these soil samples were added the various fertilizing materials according to the scheme shown in the accompanying tables. This arrangement required 18 beakers, for each of the 6 soil samples used. Six samples were left untreated to serve as checks, 6 received 100 mgm. each of ammonium sulphate, and 6 received an equivalent amount of nitrogen as oiled and hydrated cyanamid. After the fertilizers had been thoroughly mixed with the soil, water was added to bring the moisture content up to the optimum, and the moisture content was maintained at this point throughout the experiment. The usual additions of calcium carbonate were omitted in order that the laboratory conditions for nitrate formation might more nearly approximate field conditions. Enough soil samples were used

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 7
Apr. 1, 1927
Key No. H-14

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to allow for an analysis of duplicate samples at 3 different incubation periods. The experiment was started September 24, 1921, and analyses were made after incubation periods of 25, 42, and 61 days, respectively, at room temperature. The phenoldisulphonic acid method for determining nitrates was used in preference to the reduction method, because of the interference of the various decomposition products of cyanamid in the accuracy of the latter method.

RESULTS WITH SOILS FROM FIELD NO. 1

The soil in field No. 1 is a very poor hilly loam, slightly acid, especially low in phosphorus and nitrogen but capable of producing large crops of cotton when properly fertilized. Where cyanamid together with phosphates was used on this soil as a fertilizer for cotton, a marked retarding of early growth was noted, but to a lesser extent than on other fields. Usually the yields were nearly as good as with ammonium sulphate and sodium nitrate. The results of nitrification studies with this soil are given in Table 1.

TABLE 1.—*Nitrate content of soils from field No. 1 after fertilization with cyanamid and ammonium sulphate*

Treatment in field	Treatment in laboratory	Number of milligrams of nitrogen used per 100 gm. of soil	Quantity of nitrates present after 25 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 42 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 61 days (milligrams of N per 100 gm. of soil)
Unfertilized	Untreated		0.87	1.79	2.00
Do	do		.68	1.49	2.48
Average			.78	1.64	2.24
Unfertilized	Cyanamid	21	.43	.58	.58
Do	do	21	.49	.50	.58
Average			.46	.54	.58
Unfertilized	Ammonium sulphate.	21	1.10	4.33	6.93
Do	do	21	1.08	4.00	7.43
Average			1.09	4.17	7.18
Cyanamid, NH₃, 80 pounds; acid phosphate, P₂O₅, 80 pounds; potash, K₂O, 40 pounds.	Untreated		3.36	5.20	6.93
Do	do		2.89	4.84	7.43
Average			3.13	5.02	7.18
Cyanamid, NH₃, 80 pounds; acid phosphate, P₂O₅, 80 pounds; potash, K₂O, 40 pounds.	Cyanamid	21	.42	.95	6.12
Do	do	21	.49	1.04	7.17
Average			.46	.99	6.64
Cyanamid, NH₃, 80 pounds; acid phosphate, P₂O₅, 80 pounds; potash, K₂O, 40 pounds.	Ammonium sulphate.	21	3.36	10.40	18.57
Do	do	21	3.72	10.40	20.00
Average			3.54	10.40	19.28

It will be observed that the addition of cyanamid to the soil taken from the unfertilized portion of the field practically stopped nitrifica-cation and that even after a period of 61 days the nitrate content was not so great as in the untreated soil.   Under exactly the same conditions the soil receiving ammonium sulphate showed a gradual increase in nitrate content as the incubation period increased.   These results were obtained in spite of the fact that cyanamid is an alkaline material and ammonium sulphate an acid salt.   Undoubtedly, if cal-cium carbonate had been added to the soil, a much higher percentage conversion of the nitrogen in both cyanamid and ammonium sul-phate, particularly in the latter, would have been obtained.

For the sake of comparison soil was taken from an adjoining plot which for three successive years had received a complete fertilizer in which cyanamid was the nitrogen carrier.   Samples of this soil were incubated in the laboratory either without additional fertilization or with cyanamid or ammonium sulphate.   The results of these tests were somewhat similar to those obtained with soil from the unfer-tilized portion of the field, except that the nitrate content of soils and percentage nitrification of added materials was higher in practically every case.   This may be attributed to a variety of factors, but undoubtedly the presence of an abundance of available phosphoric acid and potash was the most important factor.   Furthermore, this well-fertilized soil probably had a more vigorous nitrifying flora, was less acid because of the field applications of cyanamid, and of course contained more nitrogen.   Again, the marked retarding effect of cyanamid on nitrification is brought out.   After 42 days the nitrate content of the cyanamid-treated soil was less than one-fifth of that of the control soil, and at the end of the experiment it was still not equal to that of the control.   The nitrifying power of the soil was good, however, as shown by the results with ammonium sulphate.

RESULTS WITH SOILS FROM FIELD NO. 3

This soil was a much heavier loam than that of field No. 1, more compact, located at a lower elevation, and not as well drained as is desirable.   The natural fertility was good and the yields of corn above the average, even without fertilization, but the results with cotton were very much poorer than on field No. 1.   The nitrification results on soil from a fertilized and unfertilized portion of the field are given in Table 2.

TABLE 2.—*Nitrate content of soils from field No. 3 after fertilization with cyanami⁷ and ammonium sulphate*

Treatment in field	Treatment in laboratory	Number of milligrams of nitrogen used per 100 gm. of soil	Quantity of nitrates present after 25 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 42 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 61 days (milligrams of N per 100 gm. of soil)
Unfertilized	Untreated		0.61	1.30	1.63
Do	do		.61	.96	1.86
Average			.61	1.13	1.74
Unfertilized	Cyanamid	21	.26	.34	.47
Do	do	21	.19	.30	.52
Average			.23	.32	.49
Unfertilized	Ammonium sulphate.	21	1.58	4.16	9.18
Do	do	21	1.37	4.00	8.67
Average			1.47	4.08	8.92
Cyanamid, NH₃, 80 pounds; basic slag, P₂O₅ 80 pounds; potash, K₂O, 40 pounds. Do	Untreated do		1.79 1.86	3.06 2.74	4.33 4 16
Average			1.82	2.90	4.24
Cyanamid, NH₃, 80 pounds; basic slag, P₂O₅, 80 pounds; potash, K₂O, 40 pounds. Do	Cyanamid do	21 21	.52 .58	1.44 .80	2.54 2.48
Average			.55	1.12	2.51
Cyanamid, NH₃, 80 pounds; basic slag, P₂O₅, 80 pounds; potash, K₂O, 40 pounds. Do	Ammonium sulphate. do	21 21	5.07 4.00	10.40 10.00	13.68 14.05
Average			4.54	10.20	13.86

The general trend of these results conforms very closely to that for field No. 1. Again, cyanamid failed to nitrify to any marked extent during the duration of the experiment, and in every case in which this material was used there was less nitrate present at the end of two months than in the untreated soils.

Under the same conditions, ammonium sulphate nitrogen was converted into nitrates fairly rapidly. The nitrate content of the soil taken from the plot receiving a complete fertilizer was considerably higher than the nitrate content of the soil taken from the unfertilized plot, as in the case of field No. 1.

RESULTS WITH SOILS FROM FIELD NO. 4

The soil of this field was quite poor, well-drained, and similar in physical properties to that of field No. 1. It produced very good yields of cotton and corn when properly fertilized. Without fertilization it yielded practically nothing. When cyanamid was used with the soil from field No. 4, cotton gave a fair yield, but the retarding period lasted for so long after planting that the fertilizer could not be considered as satisfactory for cotton under the conditions used. The nitrate contents of the variously treated soils, as shown by laboratory studies, are given in Table 3.

TABLE 3.—*Nitrate content of soils from field No. 4 after fertilization with cyanamid and ammonium sulphate*

Treatment in field	Treatment in laboratory	Number of milligrams of nitrogen used per 100 gm. of soil	Quantity of nitrates present after 25 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 42 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 61 days (milligrams of N per 100 gm. of soil)
Unfertilized	Untreated		0.36	0.33	0 68
Do	do		.27	.33	.76
Average			.32	.33	.72
Unfertilized	Cyanamid	21	.10	.08	.12
Do	do	21	.08	.06	.13
Average			.09	.07	.12
Unfertilized	Ammonium sulphate.	21	1.04	2.00	3.71
Do	do	21	1.00	1.49	4 16
Average			1.02	1.74	3.93
Cyanamid NH₃, 80 pounds; basic slag, P₂O₅, 80 pounds; potash, K₂O, 40 pounds.	Untreated		2.67	3.59	6.93
Do	do		3.15	3.59	6.71
Average			2.91	3.59	6 82
Cyanamid, NH₃, 80 pounds; basic slag, P₂O₅, 80 pounds; potash, K₂O, 40 pounds.	Cyanamid	21	.65	.95	1..9
Do	do	21	.52	.87	1.53
Average			.59	.91	1 51
Cyanamid, NH₃, 80 pounds; basic slag, P₂O₅, 80 pounds; potash, K₂O, 40 pounds.	Ammonium sulphate.	21	12.68	20.80	27.37
Do	do	21	13.33	19 26	28 89
Average			13.01	20.03	28 13

The results obtained in field No. 4 are practically a repetition of the results obtained in fields Nos. 1 and 3 as given in Tables 1 and 2, respectively. Without fertilization, either in the field or laboratory, the nitrate content of this soil is exceedingly small, and the nitrifying efficiency very low. Even where ammonium sulphate was added in the laboratory to soil from the unfertilized field plot, only about one-seventh of the nitrogen has been converted into nitrates at the end of two months. Under the same conditions, soil from the fertilized plot showed a 100 per cent conversion of the ammonium sulphate nitrogen to nitrates. The use of cyanamid on both the fertilized and unfertilized soils, practically stopped all nitrification during the period of the experiment. The nitrate content in such cases was never as great as on the check plots.

DISCUSSION

The most striking point brought out by the results of the experiments is the fact that in all cases where cyanamid was applied to a soil in the laboratory, the amount of nitrates present in that soil was decreased below the checks, even in the case of the longest incubation

period. It will be noticed further that the use of cyanamid in the field over a period of two or three years resulted in a slight increase in the accumulation of nitrates. When additional quantities of cyanamid were added to these soils in the laboratory, the nitrates were markedly decreased and even after an incubation period of two months the supply was in no case as great as in the same soil kept in the laboratory to which no additional cyanamid was added.

The nitrification of ammonium sulphate in the soils which had received no field applications of fertilizer was rather slight, but in all cases the nitrate accumulation was greater than in the control plots or in those which received laboratory applications of cyanamid. The nitrification of ammonium sulphate was much more rapid and complete on the soils which had received field applications of cyanamid, phosphate, and potash made for the previous two or three years. The application of the complete fertilizer, together with the lime in the cyanamid, greatly increased the nitrifying capacity of the soils.

In considering the results reported here, it is realized that the laboratory applications of the cyanamid were far in excess of those used in the field. However, they were no greater than the applications commonly used in nitrification experiments. It should be remembered also that the cyanamid was thoroughly mixed with the soil samples in the laboratory, while in the field it is commonly drilled in the row. Regardless of the rate of application, the results are certainly comparable with those obtained with ammonium sulphate and give some indication of what takes place in many instances under practical conditions.

In the field experiments with the same soils, cyanamid could not be considered as a satisfactory fertilizer for cotton. In the presence of this material, growth was slow during the first two months but usually improved to a considerable extent later. The color of the plants was always an exceedingly dark green, indicating that they were being limited largely to ammonia and organic forms of nitrogen. The nitrification results with these same soils show that the supply of nitrates is actually less during the period of two months subsequent to fertilization with cyanamid than on the corresponding check plots. Since cotton requires nitrate nitrogen for its best growth, there seems to be little doubt that its failure to form readily from cyanamid largely explains why poorer results were usually obtained with this material than with any other source of nitrogen.

SUMMARY

Experiments are reported which show that cyanamid greatly retards nitrification in soils, thus explaining partially, at least, why in previous experiments cyanamid was not a satisfactory fertilizer for cotton. These experiments with soil samples taken from the field where the cotton was growing show that this crop had a smaller nitrate supply available when fertilized with cyanamid than did the cotton on the control plots. Doubtless the presence of certain injurious decomposition products of the cyanamid play a part also. These decomposition products act in some cases as direct plant poisons, and in others merely as poisons for the nitrifying bacteria.

EFFECT OF PUMPING FROM DEEP WELLS ON THE GROUND-WATER TABLE[1]

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INTRODUCTION

The increase of pumping in* the San Joaquin Valley of California has focused attention on the need for information in regard to its effect on the ground-water supply.

Pumping in the San Joaquin Valley has a twofold purpose: (1) To supply water for irrigation and (2) to lower the water table and provide drainage. Although most of the water obtained as the result of drainage operations is used for irrigation, such economic use is only incidental to the primary purpose of the drainage pumps.

The pumping plant from which the data presented in this paper were obtained was installed in June, 1924, at Kearney Park, Calif.,[2] for the purpose of lowering the water table and providing drainage.

EQUIPMENT

PUMP

The pumping equipment consists of a 7-inch horizontal centrifugal pump, direct connected to a 20-horsepower motor having a speed of 1,160 revolutions per minute, and set in a 7-foot concrete pit. The pump operates under a total head of 25.4 feet and discharges about 1,550 gallons per minute. The pump and pit are covered with a fireproof metal house (fig. 1). The discharge is through a concrete pipe line, either to standpipes for irrigation, or into an open ditch where the water is wasted. Figure 2 shows the water flowing from the pipe line into the open ditch.

WELL

The well is 16 inches in diameter and when bored was 69 feet deep. Figure 3 shows the nature of the material encountered in the well. The quicksand at the bottom extends to an undetermined depth. The casing extends to a depth of 53 feet below the ground surface, where it is "landed" on a 10-foot clay stratum. In "developing" the well a large quantity of sand was pumped out so that there is probably a cavity of considerable size below the clay stratum.

The total depth of 69 feet is somewhat less than the usual depth of wells for the San Joaquin Valley,[3] but because of the quicksand it was impossible to go deeper without extending the casing or changing
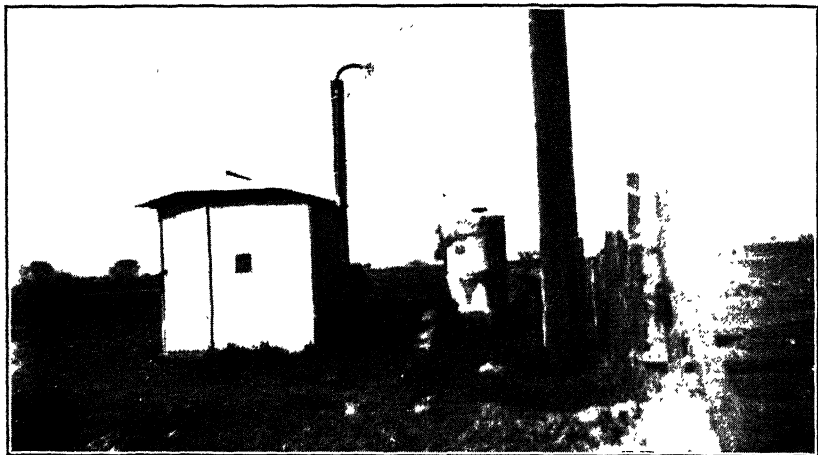
FIG. 1.—Pump house at Kearney Park, Calif. Test we No. 1 is located at the fence back o the power pole
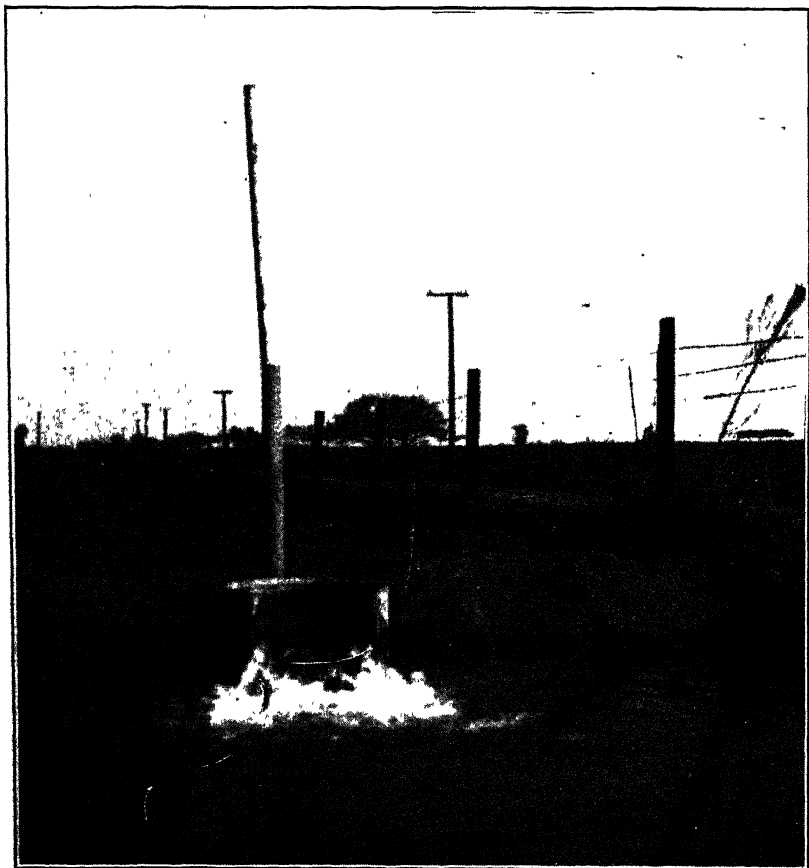
FIG. 2.—Waste pipe from the pump, showing the water entering the waste ditch

the type of casing in common use. In planning this study it was decided that a well having a capacity of 1,200 gallons per minute would be satisfactory. The actual discharge of 1,550 gallons per minute has amply justified the comparatively shallow well. This well, however, is similar in its general characteristics to the deeper wells of the valley. Its discharge could doubtless be increased by the installation of a larger pump, without either exhausting the well or creating an uneconomical draw down.

TEST WELLS

Twelve test or observation wells about 25 feet in depth were installed in lines radiating from the pump in the four cardinal directions. Figure 4 shows the locations of the test wells in relation to the pump. No. 1 is located just outside the pump house; Nos. 2, 4, 7, and 10 are 300 feet, Nos. 3, 5, and 11 are 1,000 feet, No. 8 is 1,300 feet, Nos. 6 and 12 are 2,000 feet, and No. 9 is 2,300 feet distant from the pump house. The test wells were put down with a small well-drilling outfit and are cased with 2-inch black iron pipe. The elevations in feet above sea level of the top of the casing of these wells were as follows:

No. 1, 252.70; No. 2, 252.19; No. 3, 254.02; No. 4, 252.35; No. 5, 254.96; No. 6, 253.56; No. 7, 253.48; No. 8, 250.85; No. 9, 249.91; No. 10, 252.58; No. 11, 253.58; No. 12, 256.04.

FIG. 3.—Nature of soil types encountered in drilling well at Kearney Park, Calif.

ANALYSIS OF TEST-WELL DATA

The depth to water in each of these wells was measured weekly from August 29, 1924. The test-well records for 1925 cover a complete cycle of rise and fall in the water table. As in previous findings in this locality,[4] the water table is lowest about the first of the year and highest in June. In Table 1 will be found the test-well data for the year 1925.

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[4] WEIR, W. W. GROUND WATER FLUCTUATIONS AT KEARNEY PARK, CALIF. Hilgardia 1: 133–144, illus. 1925.

TABLE 1.—*Depth* a *to water in test wells, Kearney Park, Calif., 1925*

Date	Depth in feet to water in well No.—												Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	
1925													
Jan. 2	12.9	12.3	13.0	12 8	15.9	15 4	13.0	12.3	12 2	12.7	13 4	15.3	Pump not running.
Jan 9	12.9	12.2	13.5	12 8	15.9	15.2	13.0	12.2	12.1	12.7	13.4	15.3	Do.
Jan. 16	13.1	12.4	13 8	13 0	16 1	15 3	13.1	12.2	12.0	12 9	13.4	15.3	Do.
Jan. 23	13.1	12.5	13 9	13 0	16.1	15.4	13 1	12.2	11.9	12.8	13.4	15.3	Do.
Jan. 30	13.2	12.6	13.9	13 1	16.2	15.5	13.2	12 2	11.9	12.9	13.4	15.3	Do.
Feb 7	13 0	12.4	13 4	12 8	16.0	15.4	13.0	12.1	11 9	12.7	13.3	15.1	Do.
Feb. 16	12.6	12.0	12.8	12.5	15.6	15.0	12.6	11.8	11.6	12.5	13.0	15.0	Do.
Feb 23	12.2	11.5	12 0	12.0	15.2	14.7	12.2	11.5	11.5	12 0	12.5	14.5	Do.
Mar. 4	13.6	10.5	11.4	11 6	14.1	14.0	12.6	10.6	10.8	12.5	12.0	14 0	3 hours' pumping at time of reading
Mar. 9	11.0	10.5	11.0	10.8	13.8	13 5	11.0	9 7		10.8	11.3	13.5	Irrigation around 9.
Mar. 16	10 9	10.4	11.4	10.8	13.7	13 3	10.9	10 0	9.4	10.7	11.2	13.2	
Mar. 23	16.8	13.6	12.4	14.4	13.8	13 2	15.2	10.6	9.6	15.2	13.0	13.5	Pump started Feb. 20, 1925.
Mar 30	15 1	11.9	11.9	13.2	13 9	13 2	13.7	10.5	9.7	13.7	12.2	13.3	Pump off Mar. 28 and 29.
Apr. 6	15.5	12.2	10.5	12 9		11.1	14.1	9.8	9.3	14.1	12.0	12.9	Irrigation around 9.
Apr. 13	14.3	11.6	7.8	11.5	10.1	10.4	13.0	8.3	8.8	13 1	11.0	12 2	Irrigation around 2, 3, 4, 5.
Apr. 20	13.7	11.4	9.3	11 3	11.3	10 1	12.8	8 3	7.5	13.0	11.0	11.8	
Apr. 27	14 2	11 0	9.4	11.7	11 6	10.1	12 9	7 5	6.3	13.0	10.8	11.7	
May 11	14.0	11 1	7.7	11 2	10.2	8.4	12.5	7.2	5.3	12.6	10.6	11 3	Irrigation around 3 and 6. Pump stopped May 9.
May 18	9.6		6.4		9.1	7.8	9.4	5.8	5 1	9.1	8.5	10.6	Irrigation around 2 and 4.
May 25	11 1	8.8	6 9	8.1	7.5	7.1	10 2	5.3	4.6	10.6	9 1	10.4	Pumping daytime only, May 14 to 21.
June 1	11.8	8.9	6.6	8.8	8 6	7.6	10 6	5.7	4.7	10 7	9 1	10.3	
June 8	10.7	9.6	7.5	8 6	8.6	7 4	10.3	5 7	4.3	10 9	9.3	10.4	
June 15	11 5	8.7	7.4	9.3	9.4	8.1	10.2	5.7	4.2	10 4	9.1	10 5	Pump stopped June 14 for 1 day
June 22	12.8	10.1	7.4	9 8	9.4	7.8	11.2	5.5	3.8	11.5	9 5	10.4	
June 29	13.4	10.8	7.9	10 7	10 4	8 6	11.9	6.4	4.3	12.1	10 0	10.8	
July 6	13.9	11 2	9.2	11.1	11.2	9 4	12 5	7.0		12 6	10 4	11.0	Irrigation around 9
July 13	14 6	11.8	10.4	11.8	12.0	10.1	13.0	7.4	4.9	13.1	10.9	11.5	Do.
July 20	12 1	9.2	8 4	9 4	11.6	10 4	11 2	7 5	5.8	11 2	10.3	11.8	No explanation.
July 27	14 5	11.7	9.3	11.6	12.1	10.5	13.1	8.2	6.4	12.1	11.1	11 9	
Aug 3	14.8	12 0	11.1	12.4	12 7	11.0	13.4	9.1	6 2	13.6	11.6	12.4	
Aug. 10	15 5	13 3	12.4	13 4	13 7	11.8	14 2	9.0	7.1	13.8	12.3	12 9	
Aug 17	10.3	9.7	9 9	9 7	12.5	11.8	10.1	8.8	7 6	10 1	10.9	12.7	Pump stopped Aug. 14–17, inclusive.
Aug. 24	16.5	13 4	12.9	14.0	14 1	12.4	14.9	9.7	8.0	15.0	12 9	13.4	
Aug. 31	16.3	14.2	13.6	14.3	14.9	13.1	15.0	10.2	8.5	15.1	13.3	13.8	
Sept. 7	16.6	13.9	12.3	13.7	14.6	13.2	15 2	10.4	8.9	15.5	13.5	14.1	Irrigation close to 2, 3, 4, and 5.
Sept. 14	16.0	13 2	11 3	12.7	13.5	12.6	14.6	10.0	8.9	14.9	13 0	13.9	Water in Houghton.
Sept 21	17.0	14.0	13.1	14.4	14 6	13.0	15.4	10.2	8.6	15.5	13.4	14.0	Houghton nearly dry.
Sept. 28	17 3	14 5	14 0	15 2	15 4	13 6	15 9	10.7	8 9	16.0	13.9	14.4	Houghton dry.
Oct 5	15 2	14.5	14.3	14.2	15 7	14 1	15.5	10.8	9 1	15.0	13 9	14 6	Pump off 7 hours.
Oct. 12	12.7	12 2	13.4	12.4	15 1	14.0	12 6	11.0	9 3	12.4	12 8	14.4	Pump shut off Oct. 9, 1925.
Oct 19	14 7	13.4	13.9	13.8	15 5	14.1	14.7	11.0	9 7	14.3	13.5	14.4	Pump run 40 hours, Oct. 10 to 19
Oct. 26	14 9	11 9	13.2	13.3	15 0	14.0	13.6	10.8	9 8	13.6	12.8	14 4	Pump run 7 1/2 hours during week
Nov. 2	14.3	12 4	13.7	13 5	15 4	14 2	14.2	11.0	10.0	13 9	13.2	14 4	Pump run 12 hours.
Nov. 9	12.3	11 8	13.2	12 2	15 2	14.3	12.2	11.0	10 2	12 0	12.6	14 5	No pumping
Nov 16	12 4	11 9	13 4	12.2	15 2	14 4	12.3	11.1	10 4	12 1	12.7	14.6	Do.
Nov 23	12 3	11 9	13.3	12.2	15 3	14 5	12 2	11.1	10.4	12 1	12.7	14.6	Do.
Dec 2	12 3	11 8	13.3	12 2	15 2	14.4	12.3	11.2	10 6	12 1	12.7	14 5	Rain.
Dec 7	12.3	11.8	13 3	12 2	15 2	14.4	12.3	11.1	10 6	12 0	12 6	14.5	No pumping.
Dec. 14	12 4	11.8	13.3	12.2	15 2	14.4	12.3	11.2	10 6	12.1	12 6	14.5	Do.
Dec 21	12.4	11 8	13 3	12 2	15 2	14.4	12.3	11.2	10 7	12 1	12 6	14 5	Do.
Dec. 28	12.3	11 8	13.3	12.2	15 2	14.5	12.2	11.1	10 7	12 1	12.7	14 5	Do.

a Depth in feet below top of well casing. Top of casing 1 foot above ground surface.

In Figure 5 is shown the position of the water table in relation to the ground surface, on three days in 1924. On June 4 the water table was practically a straight line through both the north and south and the east and west wells. This was the highest point recorded for that year. Measurements of the water table were taken before any pumping was done and therefore represent the normal position for that day. There is a slight slope from east to west (shown more clearly in Figures 6 and 7), and also from north
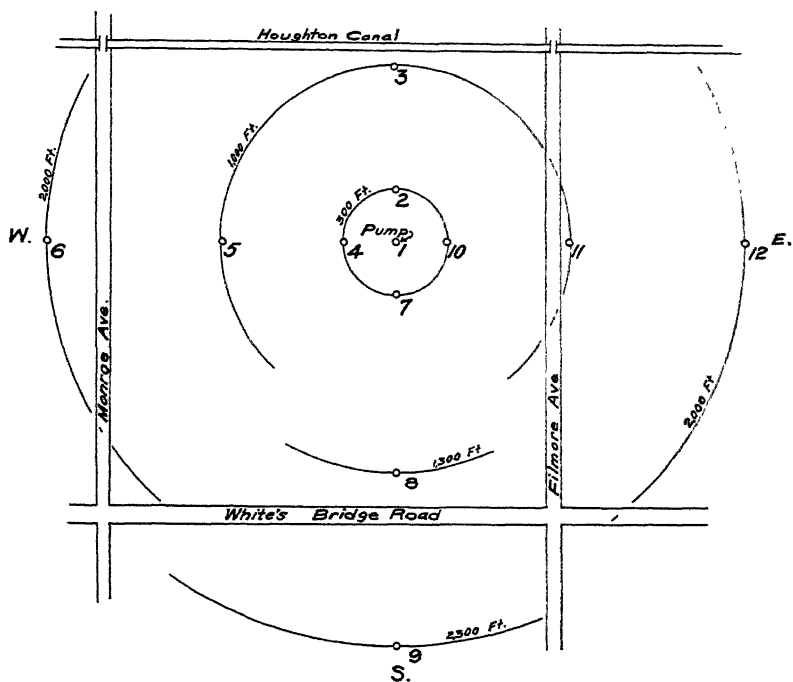
FIG. 4.—Sketch showing locations of test wells with reference to the pumping plant
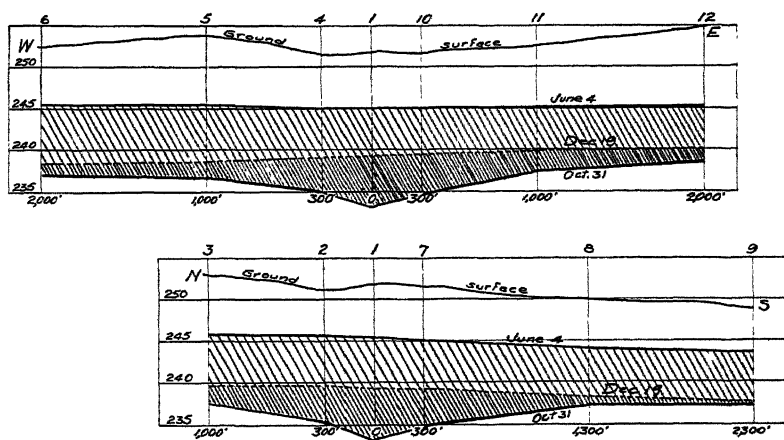
FIG. 5 —Highest and lowest water table in 1924.   On June 4 the pumping had not yet started. October 31 was the end of the pumping season and December 19 the date of the lowest water during the period when the pump was not in operation

to south. In 1924 the pumping season began August 29 and ended October 31. On the last-named date the water table stood as indicated in the diagram. On December 19, or about six weeks after the pumping season closed, the water table had reached its normal low point
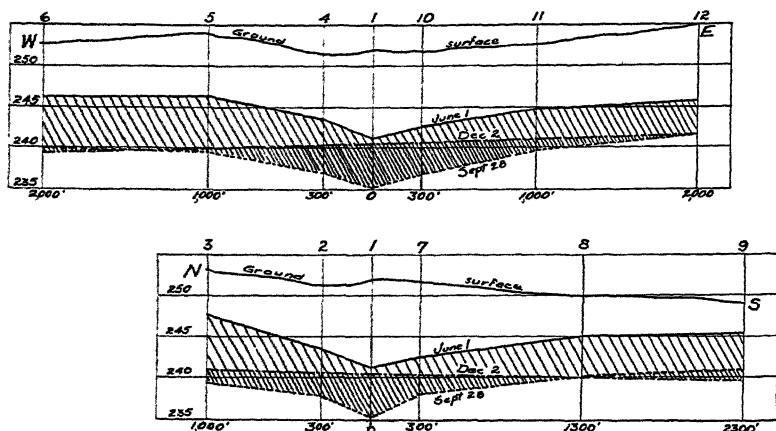
FIG. 6.—Highest and lowest water table June 1 and September 28, respectively, during the 1925 pumping season, and on December 2, several weeks after the close of the pumping season

for the winter, which, as will be observed, is above that of October 31. The lightly shaded area in Figure 5, indicating the change from June 4 to December 19, is due to the normal seasonal fluctuation and not to pumping.
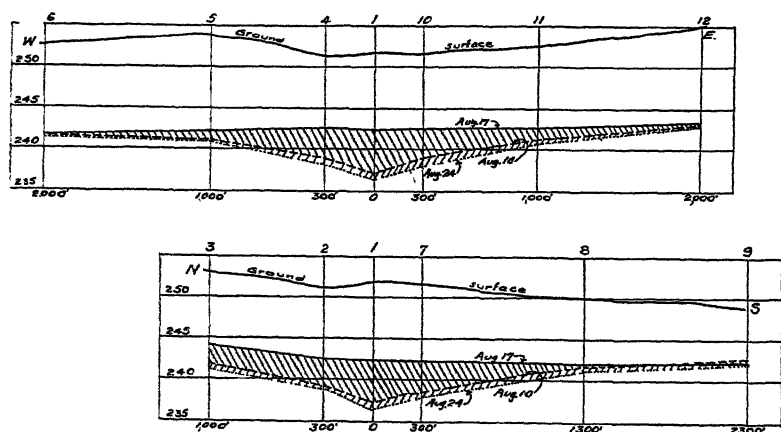
FIG. 7.—Graph shows the complete recovery of the water table in a brief period of three days during which there was no pumping

In 1925 the pump was started March 20 and was operated until October 26. There were, however, several intermissions, particularly during the early and late seasons. From May 14 to 21 the pump was operated during the daytime only. Figure 6 shows conditions for 1925 similar to those in Figure 5 for 1924. Although

by June 1, 1925, the pump had been in operation for several weeks, the water table had risen to the height indicated. The pump was operated only intermittently after September 28 and at that date the table had not reached the minimum depth.

Figure 7 shows the position of the water table on August 10, at which date the pump had been in continuous operation for several weeks. During the last three days of the following week the pump was not operated, with the result that the water table rose as indicated by the line "August 17" in Figure 7. This position is parallel to those shown in Figures 5 and 6 and is undoubtedly the normal position for that date. With the resumption of pumping for the week ending August 24, the water table again receded to a position parallel to that of August 10. This indicates that in as short a period as three days there is an entire recovery of the water table from the effects of previous pumping. The difference between the curves for August 10 and 24 is accounted for by the normal recession of the water at that time of the year.

The diagrams show that pumping has noticeably affected the water table at a distance of 2,000 feet from the pump. The normal slope of the water table toward the west and south is reversed in direction for a distance of 2,000 feet while the pump is in operation. Except for a few days in 1925, when the experimental fields were being heavily irrigated and pasture land around well No. 9 was being flooded, the tile drains with which this tract is supplied [5] contained no water, the pump having lowered the table below the tile.

In Figure 8 are shown the actual weekly fluctuations in the water table of the 12 test wells for the year 1925. Because of its proximity to the pump, well No. 1 is assumed to reflect all of the major changes in the operations of the pump. As the readings were taken only once a week, some of the minor fluctuations may be missing. It will be observed that wells Nos. 2, 4, 7, and 10, which are only 300 feet distant from the pump, show very nearly the same fluctuations; those 1,000 feet away are less affected; and those 2,000 feet away are only very slightly influenced. Although they are both 2,000 feet from the pump, wells Nos. 6 and 12 differ from each other considerably more than do wells Nos. 2 and 4, for instance, which are both 300 feet from the pump. This condition is due to other factors than the influence of the pump. Wells Nos. 6 and 12 are actually 4,000 feet apart, while wells Nos. 2 and 4 are only about 425 feet apart. Wells Nos. 6, 12, and 9 are located in areas which receive entirely different cultural and irrigation treatments. Well No. 6 is in an alfalfa field, well No. 12 is in a vineyard, and well No. 9 is in a Bermuda-grass pasture.

In the wells located 300 and 1,000 feet, respectively, from the pump there was a slight rise in the water table on October 26 that was not shown in well No. 1. The pump was operated intermittently at this season, and the readings may have been taken shortly after the pump had resumed operation and had had time to affect the water table only in the immediate vicinity.

It is interesting to observe that the final stopping of the pump in October did not affect the normal seasonal lowering in wells more

[5] WEIR, W. W. PRELIMINARY REPORT ON KEARNEY VINEYARD EXPERIMENTAL DRAIN. Calif. Agr. Expt. Sta. Bul. 273, p 103–123, illus. 1916.
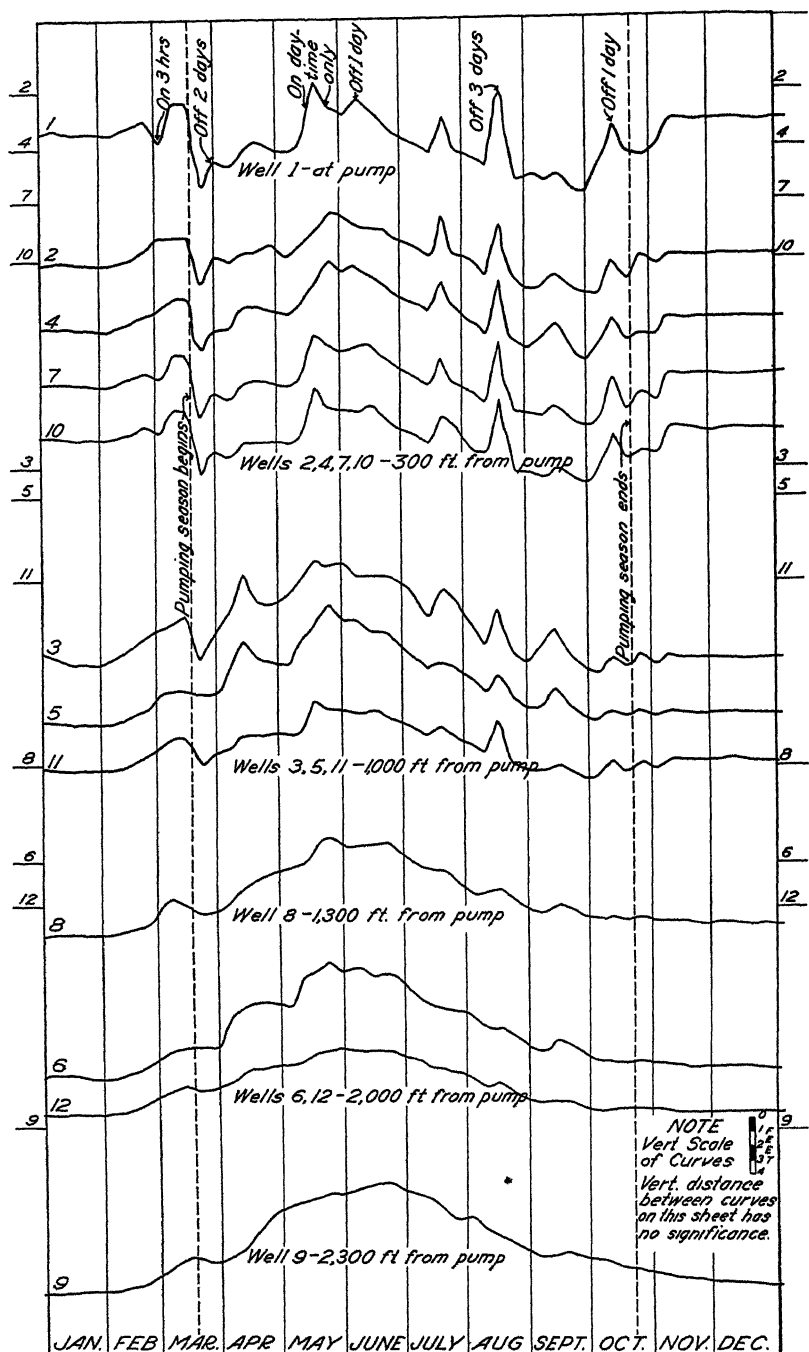
Fig. 8.—Rise and fall in the water table at different distances as the result of variations in the operation of the pump. Readings were taken weekly. Well No. 1 is assumed to reflect all of the major changes in pumping operation. Average ground surface at each well is indicated on the borders of the graph

than 1,000 feet from the pump, whereas in those within 1,000 feet of the pump there was an immediate rise to the normal position.

In order to bring out more clearly the variations in individual wells, all the curves shown in Figure 8 for any given distance are superimposed and shown in Figure 9. The water table as of January 1 is taken as a common point on each curve. The average of the
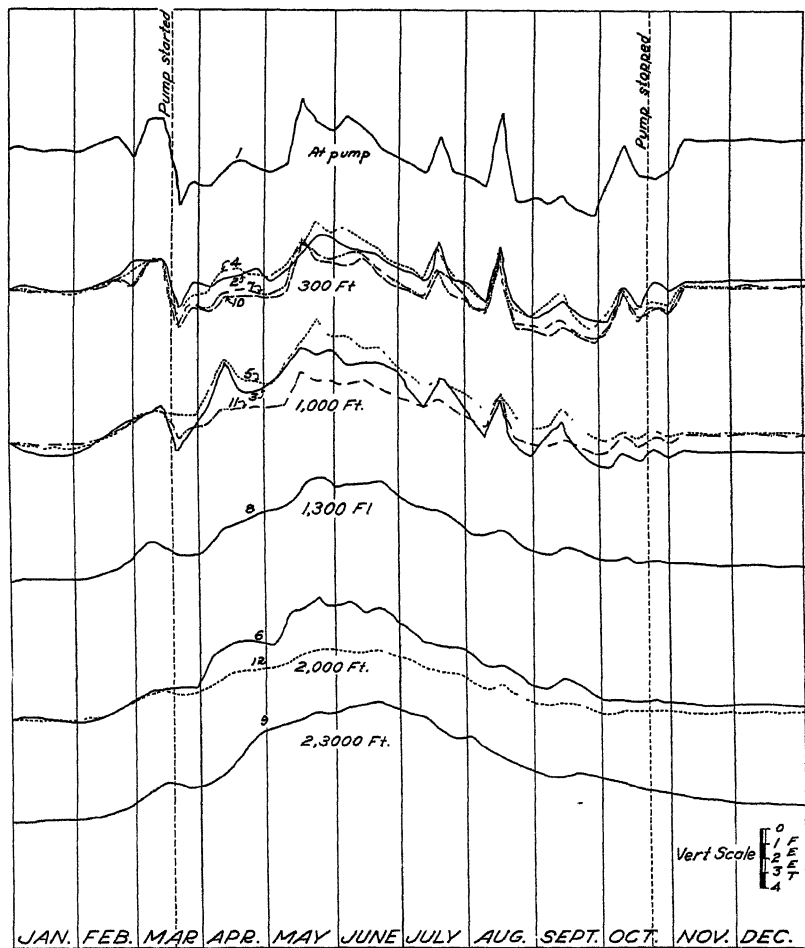
FIG. 9.—Water table for wells the same distance from the pump, superimposed. Water table as of January 1 taken as a common point

superimposed curves in Figure 9 is again superimposed in Figure 10 and shows plainly the effect of distance on the influence of the pump. Here again the water table as of January 1 is taken as a common point for all distances. The average curve for the 2,000-foot wells shown in Figure 9 is remarkably similar to the average water-table curve for the general region, as shown in Figure 5 of the writer's publication, "Ground Water Fluctuations at Kearney Park, Calif." [6]

[6] WEIR, W. W. GROUND WATER FLUCTUATIONS AT KEARNEY PARK, CALIF. Hilgardia 1:133–144, fig. 5. 1925.

Although no continuous measurements were recorded covering the discharge from this pump, a close approximation of the amount of water discharged during the year may be obtained by comparing the rating given the pump shortly after installation, with the record of the number of days it was in operation. From March 20 to October 26, a period of 210 days, the pump was in operation approximately 197 twenty-four-hour days, with an average discharge of over 1,500 gallons per minute or 1,182 acre feet for the season.

SUMMARY

The data obtained from the operation of the pump herein described is believed to be representative of that which might be obtained over a considerable portion of the east side of the San Joaquin Valley where substrata conditions are similar to those at Kearney Park. The conclusions enumerated below are applicable generally to pumps of the capacity of the one used.

Effective drainage can be obtained for a distance of more than 1,000 feet from the pump. (The writer has shown in a previous
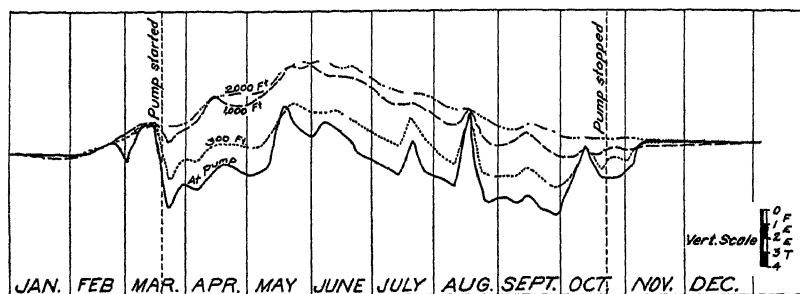
FIG. 10.—Average curves for test wells at varying distances from pump, superimposed. Water table as of January 1 taken as a common point. Each curve is the average of a group of curves (fig. 9) for the given distance

paper[7] that pumping from shallow surface wells or sumps does not affect the water table to an appreciable extent beyond a distance of 100 feet.)

In order to maintain a constant depth of water table, it is necessary to keep the pump in continuous operation during the pumping season.

There is no indication that this pump has had any permanent effect on the amount of water available for pumping. Undoubtedly, however, a large number of pumps operated continuously would permanently lower the water table, even though there was no diminution in the amount of water supplying the underground reservoirs.

There is a rapid movement of water through the water-bearing sands and gravels of the region, as evidenced by the rapid recovery of the water table to normal when the pump is stopped.

For increased effectiveness in lowering the water table and thereby providing drainage, and in supplying water for irrigation, it is likely that pumps of larger capacity than the one used in the work outlined in this discussion may be installed without either creating an uneconomical lift or bringing about a significant diminution of the water supply.

[7] WEIR, W. W. EFFECT OF PUMPING FROM A SHALLOW WELL ON THE GROUND WATER TABLE. Jour. Agr. Research 11:339-357, illus. 1917.

RECIPROCAL EFFECTS FROM GRAFTING[1]

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INTRODUCTION

It has long been recognized that grafting can effect appreciable changes in the component parts of a plant. Commenting on these changes, Jost (*2*)[3] says, "As Strasburger has found plasma bridges between stock and scion, a migration of plasma particles is not impossible." Since striking modifications in the morphology as well as in the physiology of the affected parts occur, the field for further inquiry seemed so promising as to persuade the writer to make further investigations along this line. For this purpose various reciprocal grafts of beans of different varieties and even of different species were made. The experimental work was done at the University of Illinois.

MODIFICATION OF STOCK

The extent to which grafting modifies the rootstock has been noted by several workers. Lindemuth (*3*) describes a graft of Abutilon top-worked with an annual plant, *Modiola carolina*, which was kept for three years and five months. Sahut (*8*) refers to various cases of evergreen scions, such as *Crataegus glabra* and Raphiolipsis, being grafted on the common quince, which is deciduous. He also describes a graft of the late-opening St. Jean walnut, top-worked on the common walnut, which resulted in holding back the stock for over a month. An evergreen cherry, Laurier-Amands, top-worked with a deciduous variety which was kept as long as the ungrafted deciduous stock, was also mentioned, together with early-leafing American varieties which were correspondingly retarded when top-grafted with scions of late-leafing European sorts. An extensive survey made by Vard (*9*), following the severe winter of 1890–91 in France, showed that normally hardy stocks top-worked with scions of tender varieties, such as the tea and Bourbon roses, were killed; and in no instance were such top-worked stocks as hardy as the ungrafted hardy types. Webber et al. (*5*), in observations on the freezing winter of 1913 in California, refer to the definite influence of the tops upon the stock, in grafts of citrus varieties. Not only were the tender lemon tops killed but the injury from freezing extended 3 to 4 inches down the normally hardy stock. Pomello seedlings when grafted with scions of the tender varieties were likewise injured, while the ungrafted stocks were scarcely touched.

Journal of Agricultural Research,
Washington, D. C

Vol. 34, No. 7
Apr. 1, 1927
Key No. Ill -26

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METHOD OF MAKING GRAFTS

Beans are very tender and can be grafted only while they are growing on their own roots. For this reason the approach method of grafting was found to be the most effective. The plants to be grafted were grown close together in a gallon pot. After the seedlings had straightened out, or prior to the time that the first whorl of leaves had formed, each seedling was wounded or abrased up and down the length of the stem. The two surfaces were pressed together and tied securely. To hasten union between the tissues, numerous wounds were made by piercing both stems all the way through with a needle. The callus growth which resulted made a very effective coalescence of the component tissues. After the union was established the roots corresponding to the scion were severed, thus leaving a rootstock of one variety, top-grafted with a scion of another.

LONGEVITY OF STOCK

Normally under field conditions *Phaseolus vulgaris*, the navy bean, is an annual, living about three or four months. Even where protected from freezing and maintained under the most favorable greenhouse conditions, it seldom lives more than four to five months. Under field conditions, *P. lunatus*, the Lima bean, may be kept growing until it is killed by frost. Under greenhouse and semitropical conditions, with protection from freezing, Lima bean plants can be made to live for several years. The writer has kept some for over three years in a greenhouse at the University of Illinois.

Reciprocal grafts were made between the two species for the purpose of studying the effect upon the stock. Very noticeable differences were secured with such grafts. Lima rootstocks top-worked with navy scions produced plants which lived no longer than nongrafted navy bean plants. However, by top-working navy bean rootstocks with Lima bean scions, plants were produced which had the longevity of nongrafted Lima bean plants. This shows the marked degree to which the rootstock may sometimes be modified when the plant is top-worked.

NATURE OF STARCH GRAINS IN THE STOCK

Several studies were made of the starch to determine as far as possible what changes might have occurred in the stocks of Lima and navy bean grafts. Two questions naturally arose: (1) To what extent is the elaborated material produced in the scion translocated to the rootstock, and (2) is the elaborated material modified before, during, or after such translocation?

In these studies the differences between the starch grains of the Lima and the navy bean, as brought out in Reichert's (*6*) tests, were carefully observed. In this way an effort was made to compare the starch grains of the top-worked stocks and the nongrafted plants of the two species. The differences between the starch grains were so extremely minute, however, as not to allow a definite conclusion to be drawn. Nevertheless, it seems reasonable to believe that the elaborated materials produced in the scion of a species that is quite different from the rootstock should become altered to some extent when translocated to the rootstock.

ANTHOCYANIN TRANSFER

Meyer and Schmidt (*4*) have shown that a soluble alkaloid such as nicotine will pass freely from a tobacco scion to a potato rootstock. In the case of glucosides, however, Guignard (*1*) states that there is no passage from one graft symbiont to the other. Since anthocyanin is a soluble glucoside, studies were made to determine whether or not this pigment was transferred from the tissue of one graft component to that of the other, and, if so, to what extent such a union affected either graft component.

The navy bean plants used in these experiments had a clear, bright green color and were entirely free from any anthocyanin pigment. In decided contrast, the plants of Refugee bean strain selected for the experiment were characterized by a very dark, reddish purple anthocyanin pigment.

Reciprocal grafts between the pigmented and the nonpigmented varieties showed no incompatibility whatever, as they produced well-established unions. In spite of this fact, however, the sharp line of color of the anthocyanin pigment showed where the two tissues met. Furthermore, throughout the entire life of the grafts, no indication was present of a passage of the anthocyanin pigment from the Refugee tissue to that of its graft component. Nor was there any diminution of color in the pigmented graft component. From these results it can be definitely concluded that there was no passage of the dark-colored anthocyanin from the one graft component to the other, and also that neither graft component was in any way modified in so far as pigment was concerned.

ALTERED BACTERIAL SYMBIOTIC SPECIFICITY

Another difference between the navy and the Lima bean is in respect to their specificity with a particular strain of the symbiotic-forming bacteria, namely, *Bacillus radicicola*. Normally, the strain of bacteria that is specific to one of the bean species will not inoculate the other. Studies were therefore made to determine to what extent reciprocal grafting of Lima and navy beans modifies this specificity.

Seeds of reciprocal grafts were planted and the roots of the resulting seedlings were inoculated. The offspring of the navy bean scions, top-grafted on Lima, were inoculated with Lima bean bacteria, and the offspring of the Lima bean scions, top-grafted on navy, were inoculated with navy bean bacteria. Examination of the roots showed nodules in both cases, indicating that cross inoculation was successful.

From this experiment it is evident that cross inoculation was effected with the seedlings coming from scions that had been top-grafted to the particular species to which the bacteria were specific. Thus grafting of Lima on navy beans, and vice versa, altered the specificity of the seed produced on the scion. A fuller report on this work is given by T. E. Richmond (*7*), who at the time of these experiments was a member of the division of soil biology, department of agronomy, University of Illinois, and made the inoculations on this material.

SUMMARY

The results of the investigation herein reported may be summarized as follows: (1) Reciprocal grafts can be made between· *Phaseolus vulgaris* and *P. lunatus;* (2) the longevity of the rootstock of *P. vulgaris* may become materially altered when *P. vulgaris* is top-grafted with *P. lunatus;* (3) although anthocyanin is described as a soluble glucoside, no passage of this pigment was observable in the graft symbionts between plants of anthocyanin and nonanthocyanin varieties; (4) seeds developed on reciprocal grafts of *P. vulgaris* and *P. lunatus* produced plants which could be cross inoculated, showing that an altered bacterial symbiotic specificity had been brought about.

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INFLUENCE OF FORM AND PROPORTION OF LIME USED AND OF METHOD OF MIXING ON THE RESULTING BORDEAUX MIXTURE [1]

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INTRODUCTION

Ever since Millardet (*8, 9, 10, 13, p. 734*) [2] noted that a mixture of copper sulphate and lime prevented downy mildew on grapevines in 1882, and recommended its application as a fungicide, the "copper mixture of Gironde" (*11*) has undergone numerous modifications in copper content, ratio of lime to copper sulphate, and method of preparation in the hands of different investigators. Yet the influence of the form and the proportion of lime, and of the method of mixing on the resulting Bordeaux (bouillie bordelaise) is not clearly understood and merits additional study. Space does not permit an adequate review of the literature pertaining to this subject but attention will be called to some of the earlier investigations.

REVIEW OF LITERATURE

According to Millardet and Gayon (*12*) and most contemporary writers, calcium hydroxide reacts with copper sulphate to form cupric hydroxide and gypsum; but Pickering (*1, p. 26, 27; 17, p. 1991, 1997, 2000; 18, p. 1852*) has shown that limewater added to a solution of copper sulphate precipitates a blue basic sulphate increasing in basicity toward equal mols, converted by a large excess into what is termed a double sulphate and, by still more lime, into a double oxide. The variance between the two views is essentially a difference as to the point at which the sulphate radical disappears from the resulting copper precipitate. Whether the copper precipitates are chemical entities or mixtures is difficult to determine, owing to their instability and the presence of insoluble by-products but, in the last two instances at least, the compounds designated are empirical. The reaction in more concentrated mixtures such as Bordeaux is evidently somewhat depressed. Freshly prepared copper precipitates, with the exception of the tribasic sulphate, are usually gelatinous, of a high degree of hydration, and of excellent suspension. The gypsum and excess lime in suspension substantially increase the bulk of the precipitate and the portion in solution increases the viscosity of the serum.

Experiments made from 1885 to 1890 by Ferrand (*3*), Millardet and Gayon (*14, p. 701; 15*), Gaillot (*4*), Patrigeon (*16, p. 701*), Viala and Ferrouillat (*19, p. 27*), Mach (*7*), and many others, led to a reduction in the amount of copper sulphate and more or less change in the proportion of lime. Mixtures with a smaller percentage of copper sulphate were found effective and, with a lower ratio of lime, more active and adherent. Slightly alkaline, neutral, and even acid

mixtures received attention. In this country there has been a tendency recently to revert to a higher ratio of lime with a view of preventing foliage injury.

INFLUENCE OF THE FORM AND PROPORTION OF LIME USED

The two ingredients employed in preparing Bordeaux mixture are copper sulphate and lime (calcium hydroxide), although supplementary products are occasionally added. The aim is to secure the highest possible dispersion of the resulting precipitate, as the physical properties are of paramount consideration. The copper sulphate employed in the experiments reported was of good quality and substantially free from other metals. The solution was standardized as to copper content in most instances.

FORMS OF LIME USED

Four distinct forms of calcium hydroxide were employed, i. e.: Limewater, chemically precipitated calcium hydroxide, milk of lime, and commercial hydrated lime. The limewater was prepared from diluted milk of lime by filtration, thus assuring a fairly definite amount of active calcium hydroxide, although the low content limits the concentration of the resulting spray. In the Massachusetts laboratory quickly prepared solutions contained only 0.113 gm. of CaO in 100 c. c., as determined against n/10 hydrochloric acid, using phenolphthalein as indicator. This is probably due to incomplete saturation, partial carbonation, or selective absorption of the filter paper, and possibly to all three.

The chemically precipitated calcium hydroxide was prepared by the reaction of sodium hydroxide on calcium chloride and dialyzed free from chlorides, although any other modification that assures a homogeneous suspension of fine, active particles, free from impurities, might be employed. Such a product is nearly ideal for the preparation of Bordeaux mixture but too expensive for practical use.

The milk of lime was prepared by carefully slaking a high-grade granulated "fat" lime. The objective is complete hydration with a high degree of dispersion and the formation of a smooth, creamlike product having good suspension and adhesive properties. One of the writers (Dunbar), on a trial and error basis, obtained excellent results by adding 3 volumes of granulated lime to 5 of water, mixing vigorously and gradually introducing additional water. Subsequently, 1 part of lime added to 1.6 volumes of water and diluted with boiling water was found more satisfactory. Coarse particles of lime in the milk, whether due to poor stock or method of slaking, reduce the efficiency of the resulting Bordeaux, since they serve as nuclei for the formation of larger aggregates when the lime is mixed with the copper sulphate. Straining the milk of lime through double thickness of cheesecloth was adopted as preferable to screening, but this requires more time.

As the principal difficulty in preparing Bordeaux mixture with milk of lime is in the proper slaking of the lime, commercial hydrated lime was considered as a substitute. It has undoubtedly been used by others but to what extent is difficult to say. Of the hydrated limes on the market, agricultural lime is unsuitable, but some brands of plasterers' or finishers' lime are fairly soft, bulky, and substantially free from grit; and the so-called "chemical" hydrated lime is of greater

purity and better physical characteristics. The sample of plasterers' lime employed had a volume of 2.4 c. c. per gram, 99 per cent passing a 0.5 mm. sieve, and contained 42 per cent of active lime, calculated as oxide. As dry hydrated lime disperses slowly in water it was allowed to soak overnight before it was used to prepare Bordeaux, as the suspension of the latter is materially increased thereby. Several samples of "chemical" hydrated lime received recently had an average volume of 2.6 c. c. per gram and contained 67.3 per cent of active lime (theoretical for total CaO, 75.682 per cent). The manufacturers claim 96.5 to 99 per cent will pass a 200-mesh screen.

By active lime is meant that portion which can be readily determined by titration against standard acid to the initial disappearance of color, using thymolphthalein as indicator, thereby excluding coarse particles that are slow to react. The active portion of precipitated lime and milk of lime was determined in a similar manner and all tests were based on the active and not on the total lime present. Inert material is objectionable from the standpoint of both application and spray residue.

With precipitated lime, milk of lime, and hydrated lime, the fineness and uniformity of the particles seem to determine in large measure the physical properties of the resulting Bordeaux mixture. The degree of dispersion of the lime in the three forms evidently decreases in the order named.

PROCEDURE AND RESULTS

A series of experiments with 12 different ratios of lime to copper sulphate was instituted to determine the relative activity of the four forms of calcium hydroxide and the suspension of the Bordeaux mixtures prepared with them. The same concentration of copper sulphate, equivalent to that of ordinary Bordeaux, 4-4-50, was employed except in the case of limewater where a like strength was impossible, but the ratios of lime to copper sulphate were the same. Rubber-stoppered, glass museum jars of the following dimensions were employed for the purpose. Total height, 305.5 mm. (14 inches); length and outside diameter of the body, 303.0 by 63.5 mm. (13 by 3.5 inches); and capacity to the neck, 850 to 900 c. c. The jars were graduated to 780 c. c. and the length of the column determined for each jar. Suspension is expressed in percentage. The results at the end of 1, 2, and 3 hours are reported in Table 1.

Seven hundred and fifty cubic centimeters of the limewater were added to 30 c. c. of solution containing the required amount of copper sulphate for the different ratios. In most other cases, 663 c. c. of solution containing 7.49 gm. of copper sulphate were added to 117 c. c. of lime in suspension, containing the necessary amount of active lime for the different ratios. With precipitated lime and milk of lime the demands of the two highest ratios necessitated increasing their volume to 200 and 250 c. c., respectively, and reducing the volume of copper sulphate correspondingly. In American practice the composition of Bordeaux mixture is usually designated by figures such as 4-4-50, which signifies 4 pounds of copper sulphate and 4 of quicklime in 50 gallons of mixture, and is substantially equivalent to 1 part (or 1 per cent) of each in 100. Some writers term such a mixture 1 per cent Bordeaux and indicate the proportion of lime by ratio 1 : 1. The former method is less confusing and is employed in the text. Owing to inability to control the reaction, considerable varia-

tion is inevitable in such tests. Average results therefore are reported, those tests that suffered appreciable decomposition being excluded, although by so doing a fictitious value is given those mixtures that were particularly susceptible.

TABLE 1.—*Ratio of volume of suspended Bordeaux to total volume of liquid, when different forms and proportions of calcium hydroxide were used*

No.	Bordeaux mixture	Limewater			Precipitated lime			Milk of lime			Hydrated lime		
		1 hr.	2 hrs.	3 hrs.	1 hr.	2 hrs.	3 hrs.	1 hr.	2 hrs.	3 hrs.	1 hr.	2 hrs.	3 hrs.
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	4–0.672–50	73.8	39.8	32.3	87.0	78.1	71.7	82.2	70.2	62.3	69.4	53.0	45.6
2	4–0.720–50	93.7	86.1	78.3	91.8	84.5	78.8	93.9	88.3	83.3	87.3	75.5	65.3
	Neutral:												
3	4–0.808–50	95.6	91.0	86.1	96.6	93.2	90.1	97.0	94.2	91.4	94.6	90.6	83.4
	Alkaline:												
4	4–1.000–50	92.2	84.6	77.1	97.2	94.6	92.5	97.1	94.6	91.7	95.3	91.5	87.5
5	4–1.500–50	78.8	52.3	40.6	97.6	95.4	93.3	97.3	94.7	91.9	96.0	92.1	88.7
	Basic:												
6	4–2.000–50	34.0	23.9	18.9	97.3	95.2	93.0	97.5	95.0	92.6	96.4	92.7	89.0
7	4–2.250–50	29.3	20.6	16.3	97.2	94.9	92.5	97.3	94.6	91.9	96.2	92.5	88.5
8	4–2.500–50	27.0	19.0	15.0	97.1	95.0	92.4	97.2	94.4	91.7	95.7	91.5	86.9
9	4–3.000–50	22.4	15.0	11.9	97.1	94.7	92.2	97.1	94.3	91.5	95.5	90.4	84.9
10	4–4.000–50	16.8	11.3	8.2	96.6	93.7	91.1	96.7	93.3	90.0	94.1	87.6	79.1
	Highly basic:												
11	4–6.000–50	12.5	8.0	6.2	92.1	87.3	83.4	95.1	90.7	87.0	88.1	76.9	68.2
12	4–8.000–50	9.4	6.6	4.9	89.1	83.5	79.7	94.3	90.4	86.6	84.2	72.2	63.8

With limewater the concentration of copper sulphate varied from 0.647 gm. in 100 c. c. in No. 1 to 0.054 gm. in 100 c. c. in No. 12. No. 1 gave a light blue, flocculent precipitate; No. 2 a fairly gelatinous, blue precipitate, and No. 3 a full blue, gelatinous precipitate with the highest suspension. In Nos. 4 to 12 the color and suspension gradually decreased. None of these mixtures decomposed within three hours.

With chemically precipatated calcium hydroxide the mixture 4–0.672–50 (No. 1) yielded a light blue, flocculent precipitate, increasing to a full blue, gelatinous precipitate in the mixtures 4–1–50 (No. 4) and 4–1.5–50 (No. 5). No. 5 showed a slightly higher suspension. In Nos. 6 to 12 the color and suspension gradually decreased, although the difference in suspension between the mixtures 4–0.808–50 No. 3) and 4–4–50 (No. 10) was negligible, evidently on account of the high dispersion of the lime. None of the mixtures of this series suffered decomposition within three hours.

With milk of lime the mixture 4–0.672–50 (No. 1) gave a light blue, coarse, flocculent precipitate, increasing to a full blue, gelatinous precipitate in the mixture 4–1.5–50 (No. 5), but the highest suspension was generally obtained with a 4–2–50 mixture (No. 6) or occasionally with a 4–2.25–50 mixture (No. 7). In Nos. 8 to 12 the color and suspension gradually decreased, although the difference in suspension between the mixtures 4–0.808–50 (No. 3) and 4–4–50 (No. 10) was inappreciable when the lime was properly prepared. On warm days decomposition sometimes occurred in the third hour with a 4–3–50 mixture (No. 9) and more frequently in the second or third hour with the mixtures 4–4–50 (No. 10), 4–6–50 (No. 11), and 4–8–50 (No. 12). On the average, milk of lime proved nearly as satisfactory as precipitated lime but judging from the color and suspension, was less active, far more variable, and in some instances, even erratic.

With dry, hydrated lime the full blue precipitate and the highest suspension were obtained with the mixture 4–2–50 (No. 6). The product was less efficient than milk of lime, but the mixtures 4–1–50 (No. 4) to 4–2.5–50 (No. 8) gave satisfactory results, considering the quality of the hydrate employed. Occasionally a test broke down in the second or third hour.

With all four forms of calcium hydroxide, No. 1 precipitate was of a light or greenish-blue color, and of a flocculent rather than a gelatinous character. The presence of any soluble copper was accompanied by reduced suspension. No. 2 was slightly darker in color and somewhat flocculent. Nos. 1 and 2 were rather inferior for spraying purposes. Lime in excess of the mixture 4–2–50 tends to drag down the precipitate, especially when coarse particles are present. With limewater, No. 3 was preferable, probably on account of the greater dilution.

The foregoing results conform essentially to the relative activity of the different forms of calcium hydroxide employed. Limewater is the most active, as the material is entirely soluble and partly ionized. Precipitated lime, a resultant of chemical precipitation, is finely divided and uniform and approaches limewater in activity. The activity of milk of lime varies with the quality of the quicklime and the thoroughness of slaking. The particles are naturally larger than those of precipitated lime. Commercial hydrated lime is extremely variable, both in composition and in the size and character of the particles, but it is reasonable to suppose that a high-grade product would approach freshly slaked lime in activity and assure even more uniform results in general use.

INFLUENCE OF METHOD OF MIXING

The effect of the method of mixing is important and must also be considered. There are 12 or more methods with innumerable modifications, for mixing solutions of copper sulphate and lime in suspension. At least three concentrations, i. e., concentrated, equal volume, and dilute, are feasible without subsequent dilution for each procedure, copper into lime, and lime into copper.

PROCEDURE AND RESULTS

The methods of mixing are as follows:

Method	Volume per cent	Volume c. c.
1. Concentrated copper into dilute lime	15 : 85	117 : 663
2. Equal volume of copper into lime	50 : 50	390 : 390
3. Dilute copper into concentrated lime	85 : 15	663 : 117
4. Concentrated lime into dilute copper	15 : 85	117 : 663
5. Equal volume of lime into copper	50 : 50	390 : 390
6. Dilute lime into concentrated copper	85 : 15	663 : 117
7. Concentrated copper and dilute lime poured simultaneously into a third receptacle	15 : 85	117 : 663
8. Equal volumes of copper and lime poured simultaneously into a third receptacle	50 : 50	390 : 390
9. Dilute copper and concentrated lime poured simultaneously into a third receptacle	85 : 15	663 : 117
10. Concentrated copper into concentrated lime and diluted	30 : 30 : 40	234 : 234 : 312
11. Concentrated lime into concentrated copper and diluted	30 : 30 : 40	234 : 234 : 312
12. Concentrated copper and concentrated lime poured simultaneously into a third receptacle and diluted	30 : 30 : 40	234 : 234 : 312

The volume ratios, 15 : 85 and 30 : 30 : 40, etc., are purely arbitrary, but serve to illustrate the principle involved. As in the previous experiment, rubber-stoppered museum jars were used in making the tests. The Bordeaux mixture was prepared with milk of lime from carefully slaked lime and the mixture 4–4–50 was generally employed as the basis for comparison since the mixture 4–2–50 proved less discriminating. The other forms of calcium hydroxide might also have been included, but were not considered necessary.

TABLE 2.—*Ratio of volume of suspended Bordeaux to total volume of liquid, when different methods of preparation were used*

| Method No. | Bordeaux mixture, 4-4-50, first series | | | Bordeaux mixture, 4-4-50, second series | | | | | | Bordeaux mixture, 4-2-50 | | | | | |
| | All tests | | | All tests | | | Excluding tests that decomposed within 3 hours | | | All tests | | | Excluding tests that decompose within 3 hours | | |
	1 hour [a]	2 hours [a]	3 hours [a]	1 hour	2 hours	3 hours	1 hour [a]	2 hours [a]	3 hours [a]	1 hour	2 hours	3 hours	1 hour [a]	2 hours [a]	3 hours [a]
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	88.2	57.9	50.3	92.2	81.2	73.5	93.1	84.5	76.8	93.8	88.3	82.6			
2	96.5	85.6	78.0	96.5	93.0	89.7				97.0	94.7	92.2			
3	97.2	89.3	81.5	96.4	93.0	89.8				97.3	95.1	92.7			
4	96.6	84.5	75.1	96.3	92.4	88.9				97.5	95.2	92.9			
5	95.9	81.3	72.1	96.7	93.4	90.3				97.7	95.7	93.5			
6	74.4	50.8	44.5	85.3	59.2	49.8	(b)	(b)	(b)	93.4	86.8	80.4			
7				95.4	90.2	85.2				95.9	92.1	88.5			
8	95.8	77.9	70.5	96.8	93.5	90.2				97.2	94.8	92.5			
9				96.9	93.6	90.4				97.3	95.4	93.1			
10	95.2	85.6	76.5	96.0	92.0	82.5	96.0	92.3	87.8	95.7	90.2	84.4			
11	93.1	74.8	62.9	96.2	91.4	81.8	96.3	92.7	88.4	95.7	85.8	76.9	95.9	90.5	85.6
12				96.2	85.7	76.0	96.5	93.2	89.5	95.2	79.1	73.3	96.0	91.5	86.8

[a] Blanks in this column indicate no decomposition. Results are as in "all tests."
[b] All decomposed.

In the first series reported the milk of lime was not as carefully screened in every case nor the mixtures as thoroughly agitated as in later practice. As a result the differences between the several members of the series are increased. Apparently all tests below 60 per cent suspension must have suffered more or less decomposition, but none of them were excluded from the average in this series. Of the nine methods tested, dilute copper into concentrated lime (No. 3) gave the best suspension, and dilute lime into concentrated copper (No. 6) the poorest; an equal volume of copper into lime (method No. 2) was preferable to an equal volume of lime into copper (No. 5); and concentrated copper into concentrated lime and diluted (method No. 10) was better than concentrated lime into concentrated copper and diluted (No. 11); but concentrated copper into dilute lime (method No. 1) was much inferior to concentrated lime into dilute copper (No. 4). From the above results it follows that suspension varied inversely with the amount of lime in solution when mixed. Copper into lime (methods Nos. 1, 2, 3, and 10) proved superior to the reverse procedure (methods Nos. 4, 5, 6, and 11), except in the case of concentrated copper into dilute lime (method No. 1), previously noted. Whether a small quantity of lime in solution favors the formation of a lower basic sulphate in the primary reaction or a basic sulphate of

a higher hydration, or whether the degree of fineness of the lime particles is the deciding factor by controlling the speed of reaction, or by reducing the drag on the precipitate when added to the copper solution, is uncertain; but the resulting gelatinous precipitate was more bulky and settled more slowly. Considering the data as a whole, method No. 3 gave the best suspension, with little choice between methods Nos. 2, 10, and 4 or between Nos. 5 and 8, while methods Nos. 11, 1, and 6 were the poorest. Method No. 3, requiring the addition of cold, sufficiently dilute copper sulphate to cold, concentrated milk of lime to make the required volume, and thorough agitation, was the most dependable, and as simple and practical a procedure as any.

With the elimination of coarse particles from the milk of lime (see p. 678), immediate and thorough agitation after mixing, and a tolerance of 1 per cent in rating, substantially uniform, average suspensions were obtained in the second 4–4–50 mixture series from dilute copper into concentrated lime (method No. 3), dilute copper and concentrated lime into a third receptacle (method No. 9), concentrated lime into dilute copper (method No. 4), equal volume of copper into lime (method No. 2), equal volume of copper and lime into a third receptacle (method No. 8), and equal volume of lime into copper (method No. 5); but inferior and more or less unstable mixtures were secured from concentrated copper and dilute lime into a third receptacle (method No. 7), concentrated copper into dilute lime (method No. 1), and dilute lime into concentrated copper (method No. 6), increasing in the order named; also, concentrated copper into concentrated lime and diluted (method No. 10), concentrated lime into concentrated copper and diluted (method No. 11), and concentrated copper and lime into a third receptacle and diluted (method No. 12), increasing in the order named. Dilute copper (methods Nos. 3 and 9) proved slightly superior to an equal volume (methods Nos. 2 and 8) and decidedly more efficient than concentrated copper (methods Nos. 1 and 7) whether poured directly into the lime or simultaneously into a third receptacle. Copper and lime poured simultaneously into a third receptacle (methods Nos. 7, 8, 9, and 12) was slightly more efficient than copper into lime (methods Nos. 1, 2, 3, and 10) except in the case of concentrated copper into concentrated lime and diluted, and concentrated copper and concentrated lime poured into a third receptacle and diluted (methods Nos. 10 and 12).

From the above it will be seen that the line of demarcation between dilute copper and an equal volume of copper and between concentrated lime and an equal volume of lime, from copper into lime or lime into copper, or both into a third receptacle, is not significant; but that the demarcation is apparent between an equal volume of copper and concentrated copper, and between an equal volume of lime and dilute lime by all three procedures, and also between copper into lime and lime into copper. The limiting factors noted in the first series of Bordeaux mixture, 4–4–50, probably prevail in the second, but with a more uniform and highly dispersed milk of lime their influence is more difficult to perceive. Possibly with a more highly dispersed milk of lime, if such could be prepared, these differences might in turn become even less perceptible. The rapidity with which the several mixtures decomposed is an additional characteristic to be

noted. A part of the tests by methods Nos. 10 and 11 broke down in the third hour, by No. 12 in the second or third hour, and by No. 1 in the second hour; and all the tests by method No. 6 in the first or second hour.

The results with 4–2–50 Bordeaux are higher and less discriminating than with 4–4–50, second series, except in methods Nos. 11 and 12, but otherwise serve to substantiate what has been said. A part of the tests by methods Nos. 11 and 12 decomposed in the second hour. The decomposition of Bordeaux mixture is manifested by a gradual loss of gelatinous character, more rapid settling, spurting of the mixture and the slow acquisition of a purplish color. The final dense aggregates are said to be sphaerocrystals. Decomposition increases with copper content, ratio of lime to copper sulphate, mixing of concentrated copper and dilute lime or of concentrated copper and concentrated lime and diluting, and with temperature, as previously shown.

RESULTS OBTAINED BY OTHER INVESTIGATORS WITH DIFFERENT METHODS OF MIXING

Other investigators (Table 3) have noted the effect of different methods of preparation on the suspension of Bordeaux mixture, and some of their results are cited although not strictly comparable. Butler (*2, p. 161*) found that a concentrated milk of lime (low amount of lime in solution) favored suspension but was not in agreement as to the relative efficiency of the several methods. Pickering (*1, p. 53*) showed that copper into lime was preferable to lime into copper. For some reason not apparent the writers obtained far better results on mixing concentrated solutions and diluting than Butler, Jones (*6, p. 91*), Pickering, and Warren and Voorhees (*20, p. 233*).

TABLE 3.—*Ratio of volume of suspended Bordeaux to total volume of liquid by other investigators [a] when different methods of preparation were used*

Method No.	Butler, 4-4-50		Hawkins, 4-3-50		Jones, 7.5-5-50		Pickering, 3.2-3.2-50		Warren and Voorhees, 4-4-50	
	1 hour	2 hours	1 hour	2 hours	1 hour	2 hours	1 hour	2 hours	1 hour	2 hours
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	88.5	70.9	70.3		87.0		98.0	90.0	08.2	
2	92.7	86.3			87.0		93.0	64.0		
3	94.8	89.5								
4	92.6	85.3	80.5		85.0		54.0	43.0	79.5	
5	93.9	88.7			96.0		69.0	54.0		
6	91.2	82.9								
7										
8	92.0	84.6	97.1		87.0				08.2	
9										
10	72.7	47.2					30.0	25.0		
11	76.6	54.9			48.0		27.0	22.0	22 7	
12										

[a] For specific reference to the work of these investigators see "Literature cited," p. 685.

While the fungicidal activity of Bordeaux mixture is said to be inversely proportional to the amount of lime in excess of that required for complete precipitation of the copper, its efficiency as a whole is largely dependent on the degree of dispersion and other physical characteristics as determined by suspension, which is undoubtedly the simplest and most practical method of evaluation.

SUMMARY

Four forms of lime (limewater, chemically precipitated lime, milk of lime, and commercial hydrated lime) were employed in preparing Bordeaux and allied mixtures. The activity varies directly as the degree of dispersion and decreases in the order named.

The best ratios of active lime in the several forms to copper sulphate, as judged by suspension, are as follows:

Limewater_____ 0.538 gm.–0.109 gm.–100 c. c.
Precipated lime_____ 4 pounds–1.50 pounds–50 gallons.
Milk of lime_____ 4 pounds–2.00 or 2.25 pounds–50 gallons.
Hydrated lime_____ 4 pounds–2.00 pounds–50 gallons.

The best method of mixing, similarly judged, is by pouring dilute copper into concentrated lime or the two simultaneously into a third receptacle.

Some of the better grades of hydrated lime are promising substitutes for milk of lime but require soaking before being used.

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THE MORPHOLOGICAL DIFFERENTIATION OF THE PISTILLATE FLOWERS OF THE PECAN[1]

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INTRODUCTION

Most fruit trees bear blossom buds that are easily recognized during the dormant season. Such, however, is not the case with the pecan, whose fruiting habit has been little understood because of the obscurity of the pistillate buds during the dormant season. Although pruning is a major subject in the science of horticulture, no adequate system of pruning has yet been developed for the bearing pecan tree. The reason for this lies in the general lack of knowledge concerning its fruiting habit.

Woodroof (7,*p. 139*)[3] divided the buds of the pecan into two classes—catkin buds and vegetative buds. He did not, however, make clear the identification of the fruiting buds. The purpose of the present paper is (1) to show that there are four distinctly different types of terminal buds in the pecan, (2) to point out which of these are the fruiting buds, (3) to indicate the time at which the pistillate flowers begin to differentiate, and (4) to explain the process of differentiation.

The time of differentiation of the blossom buds of all cultivated fruit trees so far studied is in the growing season previous to the time of their unfolding. This fact led Goff (*3, p. 316*) to conclude that flowers were formed in the season preceding their unfolding even though their floral parts can not be distinguished in autumn. The findings of Albert (*1, p. 417*) in the case of *Robinia pseudacacia*, however, indicate that the differentiation of flower buds in early spring is entirely possible, so far as the histological characteristics which are observable under the microscope are concerned.

METHOD OF PROCEDURE

The winter buds of the pecan are protected by a dense mass of hairs which are compressed in such a manner that they become a great obstacle to the technician.

The selection of a method of histological technic which had been successfully used by previous investigators of pecan buds was limited to that employed by Woodroof, which consisted of the free-hand sectioning of green buds (*7, p. 139*). This method was tested thoroughly. It was found to be crude, but by its use it was possible to make fair sections of 40 microns in thickness. The great quantity of hairs in the winter buds caused the sections to be irregular, and the successive arranging of sections was a slow, tedious procedure.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 7
Apr. 1, 1927
Key No. Okla.-7

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The method was improved for rough examinations by embedding the green buds in paraffin.

A systematic effort to use the paraffin method (*2, p. 107*) resulted in the development of the acetic-anhydride method, which was successfully employed in the sectioning of the woolly winter buds. With this method there was a modification, partial removal, and unfolding of the bud hairs, thus permitting the complete infiltration of the paraffin. Moreover, the bud tissues were not so hard after dehydration as is usually the case.

Buds were collected at intervals of one week, beginning on the first Monday in September, 1925. Summer buds had been examined previous to this time to make sure that differentiation had not taken place. Ten buds were selected from different parts of the key tree, which was of the Moneymaker variety. The age of this tree was 11 years, its diameter at the base was 7 inches, its spread was 20 feet, and its height was 22 feet. As soon as differentiation began to take place in the key tree, the collections were made at three-day intervals and the studies were enlarged to include the Burkett, the Indiana, and the Halbert varieties.

After the development of the acetic-anhydride method the laboratory work resolved itself into the routine of carrying up the buds, sectioning, staining, mounting, and the examination of the sections.

A marked growth in the buds began on March 20, 1926. The embryonic shoot advanced beyond the bud hairs. By careful dissection under a binocular, it was found possible to remove all of the hairs. The dissected buds were then killed in Bouin's fluid (*2, p. 29*) and fixed in alcohol during the washing and dehydrating process.

A rotary microtome was used for sectioning. The sections were cut from 12 to 50 microns in thickness. The best results were obtained from 25-micron sections. Mayer's albumin fixative proved to be useless for these sections. Land's gum-arabic fixative (*2, p. 119*) was resorted to with success.

The technic used in fixing and floating out the ribbons consisted of smearing three drops of a 1 per cent solution of gum arabic on the slide, placing the ribbons, adding several drops of a fresh, weak solution of potassium bichromate, warming the slide high above the flame of a Bunsen burner until the ribbons floated out smoothly, then draining off the excess solutions and drying the slide in sunlight. The paraffin was removed from the slide and sections by placing them for 10 minutes in a Coplin jar of 100 per cent xylene. The slides were then passed through a 50 per cent solution of xylene in absolute alcohol to 100 per cent alcohol.

Saffranine and Delafield's haematoxylin were used both singly and as a double stain. For the alcoholic saffranine stain the slides were passed from 100 per cent alcohol through 95 per cent and 85 per cent alcohol for three minutes each before they were transferred to the stain. When the double stain was used the slides were stained in saffranine for 12 hours, passed into 50 per cent alcohol until the desired color was obtained, and then rinsed in water for 5 minutes. From water they were placed in Delafield's haematoxylin for 20 minutes, rinsed in water for 5 minutes, passed through water slightly acidulated with hydrochloric acid for 2 seconds, and rinsed again in water for 20 minutes. The slides were then passed through 50 per cent and 85

per cent alcohol for 1 minute each, through 100 per cent alcohol for 5 minutes, through 100 per cent xylene for 3 minutes, and then to balsam, after which cover glasses were applied.

As soon as the slides were dried sufficiently to prevent the slipping of the cover glasses, microscopic examinations were made, using the 8-mm. objective for preliminary studies and the 16-mm. and oil-emersion objectives for more detailed studies. As each slide was examined those sections which contained details of interest in the study were marked by means of a circle made on the cover glass with a wax pencil. This marking saved time in the final examinations and in the photographic work.

Representative slides were selected to show chronological stages in the morphological differentiation of the pistillate flowers.

TYPES OF TERMINAL BUDS

The first requirement for intelligent procedure in the study of pistillate flower buds is to ascertain exactly where these flowers occur. Woodroof (*7, p. 139*), after placing all pecan buds in two classes—catkin (reproductive) buds and vegetative buds—then pointed out that the upper serial buds are the catkin buds. It is quite true that all upper serial buds produce catkins, but all upper serial buds do not normally bear pistillate flowers. Abnormal conditions, such as the removal of certain terminal buds or the killing of terminal buds by cold, may cause lateral buds, which normally would produce catkins only, to develop pistillate flowers. Whether a pistillate flower will develop from a lateral bud under these circumstances seems to depend on some internal nutritive combination. This may be the carbohydrate-nitrogen relation of Kraus and Kraybill (*5, p. 85*). Lateral buds, which by microscopic examination are known to contain catkins, when budded into a young stock or a vigorous sprout normally produce a vegetative shoot terminated by a strictly vegetative terminal (rarely catkins develop along with the vegetative shoot). This fact has been observed even in the case of current-season buds placed in August and forced out immediately. A pistillate flower, however, is seldom if ever formed from such buds.

Pistillate flowers are usually said to originate from terminal buds. Bearing pecan trees, however, were found to have four distinctly different types of terminal buds. The first type is strictly vegetative and is the only type to be found in a young unbearing tree. It is a true terminal bud, and is distinctly different in size, shape, and structure from all other pecan buds. The second type of terminal bud is very similar in size, shape, and structure to the lateral buds. It was subtended in the growing season by a full-sized leaf. In early spring it was accompanied by a cluster of pistillate flowers which failed to develop, thus permitting the bud to occupy a strictly terminal position. Woodroof (*8, p. 678*) considers this the apical, dormant, lateral bud. From a strictly botanical standpoint this consideration is correct, but the position of the bud gives it dominance over other lateral buds and justifies its being termed a false terminal bud. The third type is a terminal bud located at the base of the peduncle of the nut cluster. During its development this bud was subtended by a full-sized leaf. It differs from the second type in

that the flower cluster set and matured a cluster of nuts. It, too, may be called a false terminal bud. The fourth type is a false terminal bud which seems to be borne on the peduncle of the nut cluster, but it was not subtended during the growing season by a fully developed
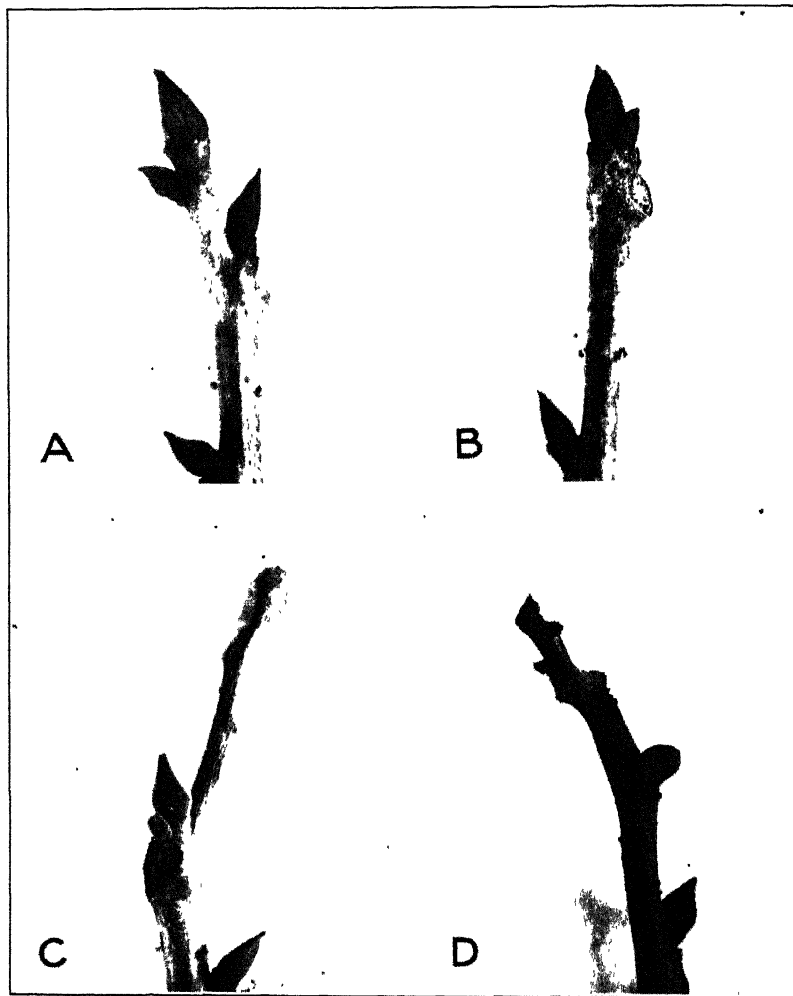
Fig. 1.—Types of terminal buds in the pecan: A, type 1, vegetative (true terminal); B, type 2, non-fruiting branch terminal (false terminal); C, type 3, fruiting-branch terminal with leaf scar (false terminal); D, type 4, fruiting-branch terminal without leaf scar (false terminal)

leaf. As many as five such buds, fully developed but without subtending leaves, have been found on a nut-cluster peduncle of the Schley. These four types of terminal buds are illustrated in Figure 1.

The relative frequency of the four types of terminal buds of the pecan tree used in this investigation is shown in Table 1.

TABLE 1.—*Frequency distribution of the four types of terminal buds on an 11-year-old pecan tree of the Moneymaker variety*

Type	Number of buds	Percentage
1, vegetable terminal	184	46
2, nonfruiting-branch terminal	76	19
3, fruiting-branch terminal with leaf	58	14.5
4, fruiting-branch terminal without leaf	82	20.5

The source of each branch which bore these terminals is shown in Table 2.

TABLE 2.—*Source of branch on which each type of terminal bud was borne*

Type of terminals on past-season branches	Kind of buds, developing branches	Number of branches developed	Number of current-season branches containing terminals of—			
			Type 1	Type 2	Type 3	Type 4
Type 1	Terminal	46	46	None.	None.	None.
	Lateral	206	116	46	18	26
Type 2	Terminal	50	None.	12	14	24
	Lateral	34	12	4	12	6
Type 3	Terminal	12	None.	2	6	4
	Lateral	30	6	8	6	10
Type 4	Terminal	None.	None.	None.	None.	None.
	Lateral	22	4	4	2	12

SOURCE OF PISTILLATE FLOWERS

That the chief source of shoots bearing type 1, vegetative terminals, is from the terminals and the laterals on a vegetative shoot is clearly shown by Table 2. It is also clearly shown that type 2 terminal buds and lateral buds near the tip of the same shoot are prolific sources of pistillate flowers. Another prolific source is the lateral buds near the tip of type 1 terminals. As a rule, fruiting branches do not originate from any lateral buds which are more than 2 inches from the terminal, regardless of its type. Not more than three pistillate buds normally develop on any one shoot. In old trees it was observed that pistillate flowers more often originate from shoots bearing type 3 and type 4 terminals, but the type 2 terminal is an important source in these trees as well.

When a pecan tree becomes 35 or 40 years of age it does not normally bear many, if any, type 1 terminals. In these trees the types 2, 3, and 4 terminals are abundant. Type 1 terminal, being the only one present in young unbearing trees, is gradually eliminated until all of the terminal buds of the tree are of the fruiting type.

This relationship of age to the source of pistillate flowers is shown in Figure 2. Since it is seldom that a branch bearing type 2, 3, and 4 terminals will produce a strictly vegetative shoot, these three types are plotted as sources of pistillate flowers; that is, fruiting buds as compared with the strictly vegetative type 1 terminal.

The identification of the pistillate flowers during the dormant season is indefinite in all features other than location. After the

buds begin to grow in spring those bearing pistillate flowers may be recognized usually by their greater length, provided they are located in the proper position. This, however, will not differentiate between the pistillate flowers originating from laterals on a vegetative shoot and vegetative shoots originating from the same source. Figure 3 illustrates the pistillate flower buds in early spring.

It has been observed that when conditions are unusually favorable to the development of pistillate flowers, even the type 1 terminals are prolific sources of them. This, however, is considered an abnormal condition.
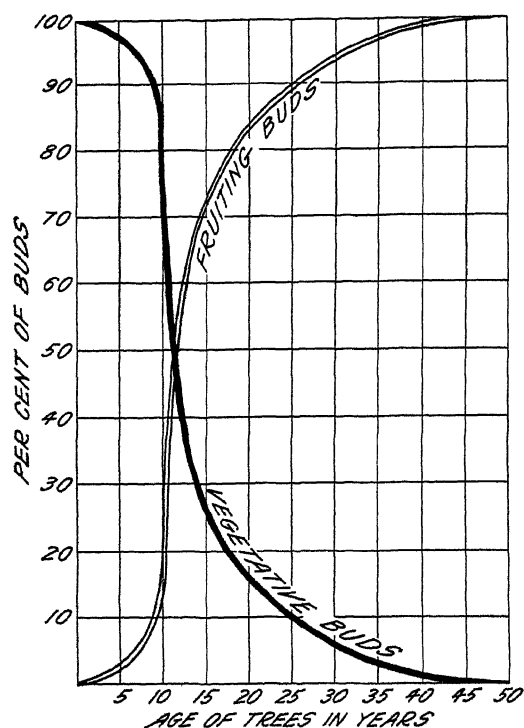
FIG. 2.—Relationship of the age of a pecan tree to the vegetative and the fruiting buds which it bears

TIME OF DIFFERENTIATION

Observable differentiation in the pistillate flowers of the pecan does not take place until after growth has started in the spring. The time of differentiation will therefore vary with seasonal, climatic, and latitudinal variations.

No differentiation had begun in the Moneymaker variety at Stillwater previous to March 25, 1926 (pls. 1, 2, and 3), although on that date the buds were swelling rapidly as a result of the unusually dry and warm weather. A marked thickening of the axis, corresponding to that described by Kraus (*4, p. 5*), was noted at this time. (Pl. 3, D.) These buds, however, were all killed by a series of freezes which came afterwards. Later, basal buds which were forced out as a result of the freezing of the terminal and upper lateral buds differentiated pistillate flowers. Halbert buds procured from Ardmore, Okla., on April 5, 1926, were showing the first signs of differentiation by the production of small blisterlike protuberances on the meristem. (Pl. 4, A.)

The first evidences of pistillate-flower differentiation in the Indiana and the Halbert varieties at Stillwater appeared on April 19, 1926. (Pl. 4, D.) This wide difference in the time of differentiation of the same variety in two localities of a State is in marked contrast with the close agreement in time of differentiation of pear and apricot buds in various environments (*6, p. 881*).

Representative section through a pistillate flower bud on different dates: A, September, 1925; B, October, 1925; C, November, 1925; D, December, 1925 (a leaf primordium is developing in this bud). All × 160

Representative section through a pistillate flower bud: A, January 1, 1926; B, January 15, 1926; C, February 1, 1926; D, February 14, 1926. All × 160
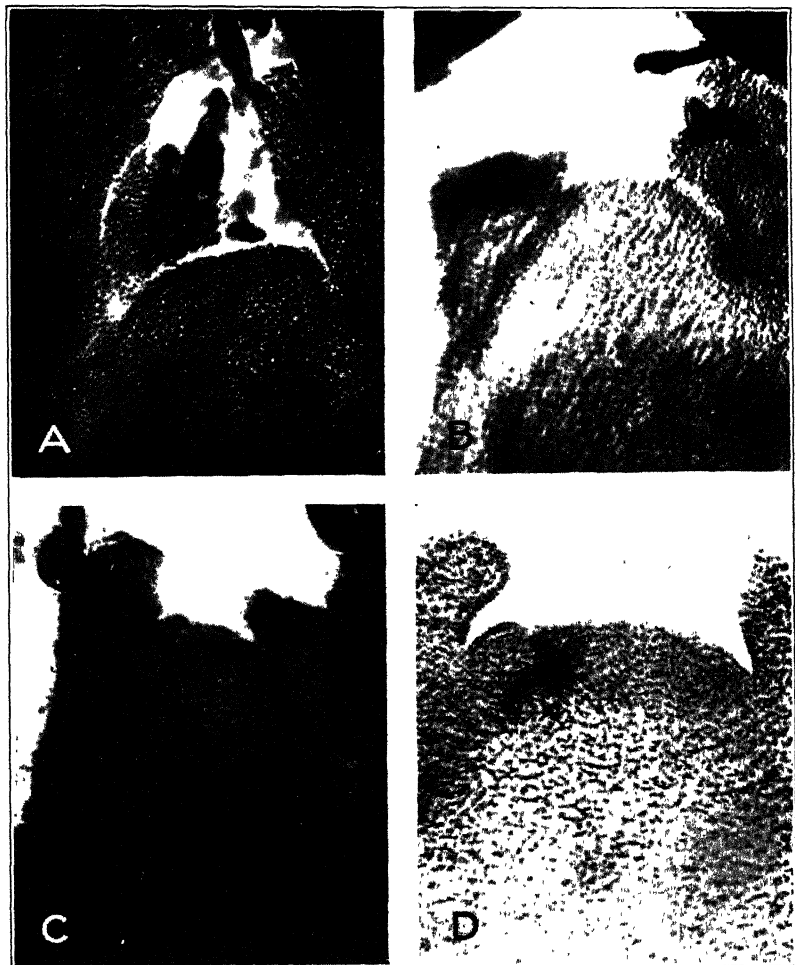
Representative section through a pistillate flower bud: A, March 2, 1926 (leaf primordium developing); B, March 7, 1926; C, March 18, 1926; D, March 25, 1926 (note the thickening of the axis; this, the first indication that differentiation is about to take place, is characteristic of the pistillate flower buds collected on this date). All × 160
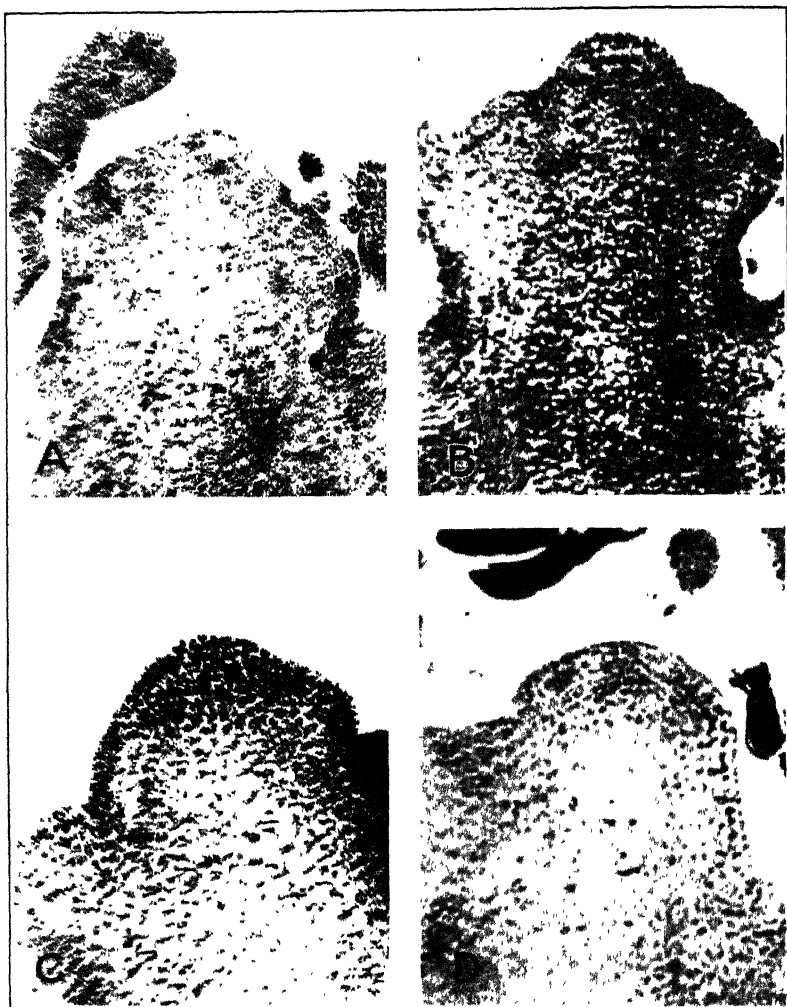
Section through pistillate flower buds of four varieties of pecan: A, Halbert bud from Ardmore, Okla., showing clearly the blisterlike protuberances which are the primordia of pistillate flowers, April 5, 1926; B, a Burkett bud from Ardmore on April 9, 1926, development advanced probably five days; C, a Moneymaker bud from Ardmore, on April 9, 1926, showing the first signs of differentiation; D, an Indiana bud from Stillwater on April 19, 1926, showing the first signs of differentiation. All × 160
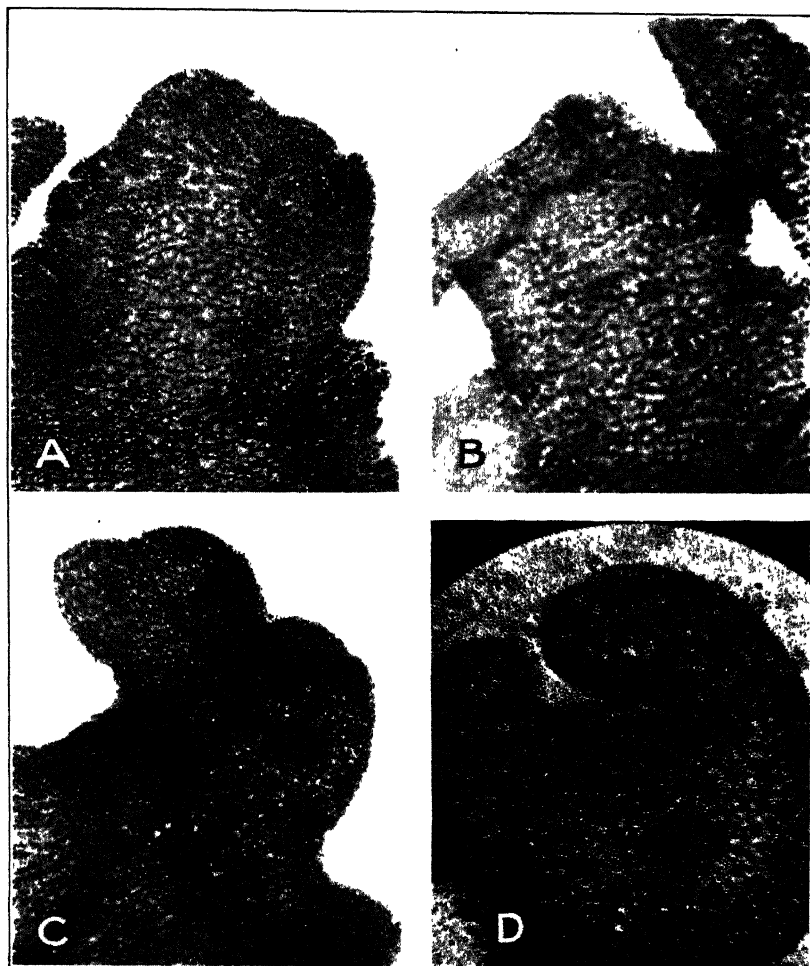
Section through pistillate flower buds of pecan, showing different stages of development:
A, Halbert bud in first stage of development, × 160; B, Halbert bud in second stage of
development, × 160; C, Halbert bud in third stage of development, × 160; D, section through
a single pistillate flower in third stage of development, × 420

A Burkett bud, showing the developing flowers. Note the narrow, necklike arrangement of the fibrovascular bundles. × 216

Woodroof (*7, p. 159*) concluded, apparently without actual observation, that differentiation of pistillate flowers takes place in early spring. In his later work (*8, p. 679*) he referred to this conclusion but offered no evidence to show that the first stages of pistillate-flower differentiation had been observed.

PROCESS OF DIFFERENTIATION

Each fruit bud of the pecan bears an average of 12 leaves. During the fall and winter there may be found from 4 to 8 embryonic leaves, varying from those definitely formed to the small leaf primordia which are conical protuberances arising from the margins of the meristem.

In late winter and early spring, bud growth progresses slowly. This growth, as was observed by Woodroof (*7, p. 139*), consists chiefly of the enlargement of the catkin groups. Leaf primordia continue to develop even after the casting off of the bud scales. In the spring, when growth has so far progressed that the total length of the bud is approximately 1 cm., the necessary leaves (12 as a mean) will have formed.

The leaf primordia are arranged in 3–2 cycles about the axis of the stem. The embryonic twig is therefore telescoped together in a compact manner. This arrangement is diagrammatically shown in Figure 4.

It is at this time that differentiation takes place. The pistillate flower buds indicated in Figure 3 are in the first stages of differentiation. The first observable indication of the pistillate flower is a cluster of blisterlike,

FIG. 3.—Two pistillate flower buds developing on the stem of a branch which bore nuts the previous season. Note how much more fully developed these buds are than the other buds on the shoot

blunt protuberances which form on the meristem. (Pl. 4, A.) After this initial step in differentiation, the development of the pistillate flower is greatly retarded. The leaves, however, are accelerated in their development and the internodes between them develop concurrently.

In approximately two weeks from their first appearance the blunt floral primordia become distinctly cup-shaped. The depression is formed by the rapid elevation of the rim and a corresponding latency in the development of cells near the center of the blisterlike protuberances. (Pl. 5, C and D.) As this cup continues to develop

FIG. 4.—Arrangement of a pecan bud at the time that differentiation of the pistillate flowers is taking place
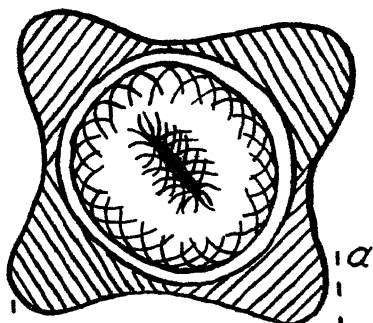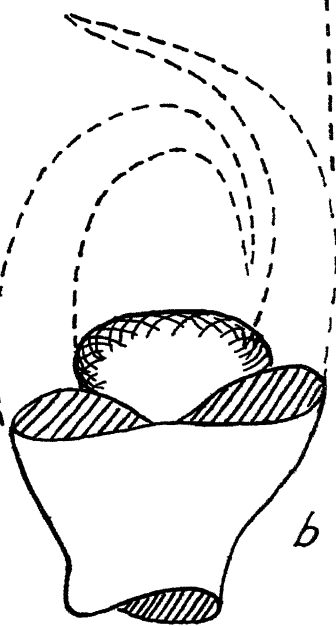
FIG. 5.—A, section through pistillate flower primordia in various stages of development; B, top view of a pistillate flower, showing the developing stigma three weeks after stage 1; b, side view showing the arrangement of the bracts

it becomes the hull of the embryonic nut. The cavity broadens and a second depression forms in the center, as is shown in stage 4 of Figure 5. Four points on the rim of the first cup develop more rapidly than the remaining part. These become the bracts of the calyx. As they develop they fold over and protect the stigma which forms around the second depression. This depression becomes a cavity in which the ovule develops, as is indicated in stage 5 of Figure 5.

The fibrovascular bundles of the stem continue through the developing peduncle to the pistillate flowers. A distinct necklike arrangement of these bundles is observable even in the early stages of differentiation. (Pl. 6.)

SUMMARY

There are four distinctly different types of terminal buds in the pecan. Type 1 is a true terminal; types 2, 3, and 4 are false terminals.

Normally, pistillate flowers are produced in terminal buds of types 2 and 3 and in lateral buds near the end of shoots bearing terminals types 2, 3, and 4. Lateral buds on shoots bearing type 1 terminals may produce pistillate flowers. In certain exceptional circumstances type 1 terminals may produce pistillate flowers.

Observable differentiation in the pistillate flowers of the pecan does not take place until after growth has started in the spring. The time will therefore vary with seasonal, climatic, and latitudinal conditions. Some difference has been noted between varieties, but this difference is not as great as that which results from different latitudinal locations of the same variety.

The rate of development of the pistillate flowers is much slower than that of the leaves on the same shoot. Five stages of development have been traced under the microscope.

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A CYTOLOGICAL STUDY OF ORANGE LEAF RUST, PUCCINIA TRITICINA PHYSIOLOGIC FORM 11, ON MALAKOFF WHEAT [1]

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INTRODUCTION

This paper reports the second of a series of cytological studies of orange leaf rust, *Puccinia triticina* Eriks. physiologic form 11, on wheat. The first (*4*) [3] reported a study of this strain of rust on a fully susceptible variety, Little Club; the present paper treats of a consistently resistant host, Malakoff; and the third is to deal with leaf-rust infections on Kanred wheat, which is variable in its reaction to this rust.

MATERIAL AND METHODS

Seed of Malakoff, C. I. 4898, was received from E. B. Mains April 22, 1924. The strain of leaf rust is the same as that used in the studies of this rust on Little Club. It was collected by W. W. Mackie at Eureka, Calif., in 1923, purified by making a single-spore culture, and carried on in the greenhouse. A specimen sent to E. B. Mains was identified as *Puccinia triticina* physiologic form 11.

Plants for cytological study were grown in the field. Seed was sown April 25, 1924, and the seedlings were inoculated May 4. The second lot was sown January 17, 1925, and the seedlings were inoculated February 26. For older material, seed was sown June 1, 1924, and the half-grown plants were inoculated January 14 and again February 19 of the following winter. The plants were kept covered with a bell jar for 48 hours after inoculation. Material was fixed at intervals from the time of inoculation until 18 days later.

The ordinary cytological technique was modified in a few details. Chrom-acetic-urea mixtures (usually 1 per cent chromic, 1 per cent acetic, and ½ per cent urea salts) were used as fixing fluids. Material fixed in the cold at 40° F. proved more satisfactory than material fixed at room temperature. In embedding, the pieces of infected leaf were placed in the interior of the paraffin block and not allowed to sink to its lower surface. This was particularly necessary in

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 8
Apr. 15, 1927
Key No. G-5€4

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working with the tougher leaf tissue of grown plants, as it prevented tearing out during sectioning. Older leaf tissue is likely to break in sectioning, but by cutting exactly parallel to the veins, sections between one vein and the next can be obtained in good condition. The triple stain was used unless otherwise stated.

INVESTIGATIONS

Malakoff wheat is highly resistant to *Puccinia triticina* physiologic form 11 at all ages and under all the environmental conditions tested. Macroscopic evidence of infection is slow in appearing. A few small greenish flecks show about the sixth day in plants grown in May. These increase slowly in size and number, usually turning bright yellow. They remain less than a millimeter in diameter. In winter, the flecks form still more slowly. They do not appear for 8 or 10 days, and the great majority are less than one-fourth of a millimeter in size. Uredinia are rare and are of almost microscopic dimensions.

The three sets of rusted plants were grown at different seasons of the year and encountered different weather conditions. Cytological study shows points of difference.

The first set was planted in the field April 24, 1924, after an exceptionally dry season, and there was no rain during the growth of the seedlings. The plants were exposed to good light (71 per cent of the possible sunshine). They were watered just enough to prevent wilting.

Microscopic study showed that the spores germinated vigorously and that the germ tubes entered the stomata of the host promptly. Of 66 fungi which formed appressoria on the stomata, 60, or 91 per cent, had entered the host by the second day. Under these conditions, at least, the fungus enters Malakoff leaves freely.

The process of entry is not altered in any way by the fact that the host is resistant. It resembles closely the entry into a susceptible host (4).

Steps in this process are seen in Plate 1, A, B, and C. Two days after inoculation, as noted above, spores had germinated, the appressoria had formed, and the majority of the fungi had passed through the stomatal slit, formed the round substomatal vesicle, and pushed out the infecting hypha. A few were still at the stage represented in Plate 1, A. The drawing represents half of a guard cell, *b d*, and the fungus on it. The fungous plasm has passed between the guard cells and formed the substomatal vesicle, *c*, leaving the appressorium, *a*, empty and collapsed. Entry commonly takes place, as in this case, through one end of the stomatal slit. Six nuclei are seen in this section of the vesicle. The total number probably is eight.

EXPLANATORY LEGEND FOR PLATE 1

A.—Portion of guard cell, *b d*, bearing the empty appressorium, *a*, on its outer side and the substomatal vesicle, *c*, within. ×730

B.—Two-day infection. The substomatal vesicle, *a*, is in contact with the accessory cell of stoma, *e*. The infecting hypha, *b*, contains six nuclei. Haustorium mother cell, *c*, at its tip is wedged between two mesophyll cells. ×730

C.—A slightly later stage, also from a two-day infection. The haustorium mother cell is producing a young haustorium, *a*. The host nucleus, *b*, is near by. ×730

D.—Eight-day infection. The fungus has been dead several days. There is an empty swollen hypha at *a*, a dead host cell at *b*, and swollen walls *b*, *d*, and *e*, in contact with the dead cell. There is very little disturbance in the surrounding tissue. ×333

E.—Exceptionally large eight-day mycelium (over 300µ in diameter) with empty appressorium, *a*, and vesicle, *b*. The stoma is dead. The central mycelium is drained, but hyphae further out, *i* and *j*, contain cytoplasm. Haustoria at *c* and *f* have expanded partly. In cells *d* and *e* the contents have dissolved, leaving the cells empty. Host nuclei, *g*, die prematurely. Walls in contact with dead cells, as at *h*, may become swollen. ×333
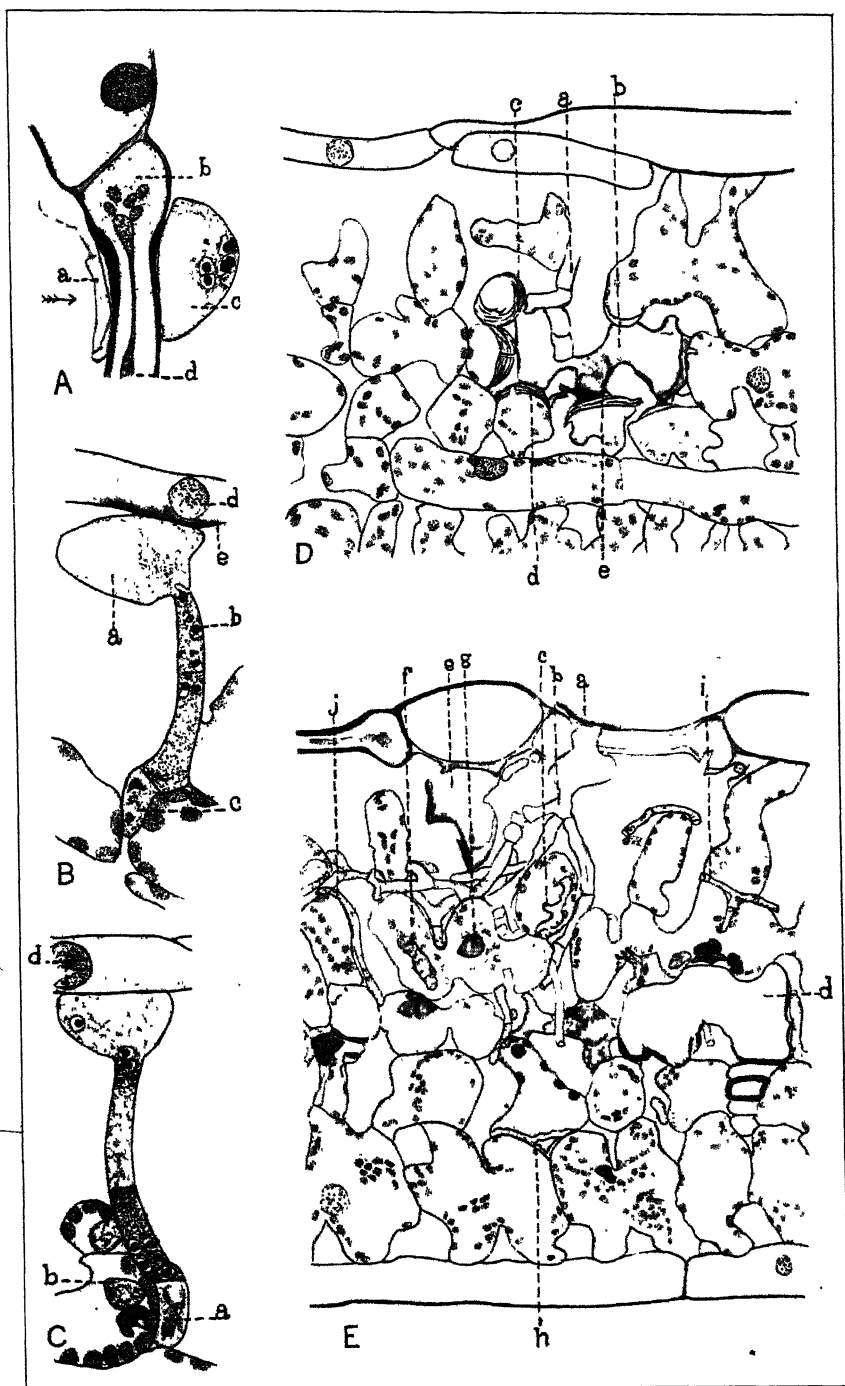
Puccinia triticina physiologic form 11 on Malakoff wheat   Infections on seedlings grown in dry bright weather, in April and May, 1924

For explanatory legend see page 698
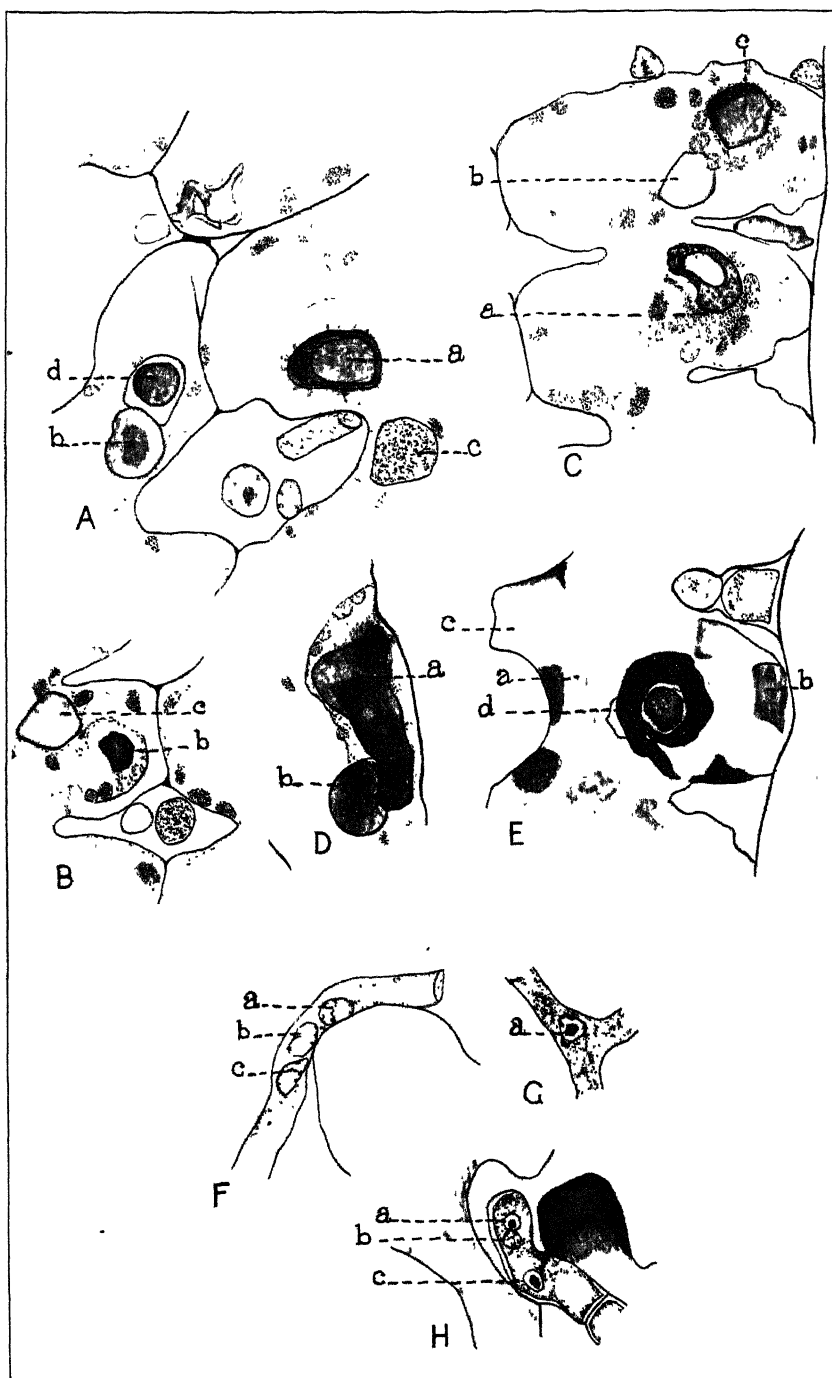
Puccinia truticina physiologic orm 11 on Malakoff wheat. Infections on seedlings grown in dry bright weather, in April and May, 1924

For explanatory legend see page 699

From this rounded, more or less hemispherical vesicle, the infecting hypha pushes out. No case has been noted in which more than one hypha forms from the vesicle. This hypha does not run over the end of the stoma and keep in contact with the inner surface of the epidermis, as is so commonly the case in stem rust, but instead it dips straight down into the large air space below the stoma.

In Plate 1, B, the vesicle, *a*, has pushed out the infecting hypha, *b*, which first encountered a mesophyll cell, then soon became wedged between two cells, and formed the short terminal haustorium mother cell, *c*. The hypha contains six or more nuclei, and the mother cell probably three. Plate 1, C, shows a slightly more advanced stage, and a young haustorium is forming at *a*. A portion of the host nucleus (divided in sectioning) is seen at *b* in contact with the haustorium. In both B and C the section was oblique and cut through an accessory cell of the stoma. It is noteworthy that in both of these accessory cells the host nucleus, *d*, lies at the point in the cell nearest to the fungus, as if attracted by it, and in the one at *e* the host-cell wall is injured (the injury is represented by shading). Even at this stage it is not uncommon to find a stainless area in guard-cell walls in contact with the fungus, which shows that these walls are altered chemically by fungous secretions.

In only one or two cases in the two-day material was the host cell in which a haustorium had formed killed. In four-day material, however, cells invaded by the fungus were killed and collapsed, and are seen in the preparations as irregular shrunken red-stained masses.

The formation of germ tube, appressorium, vesicle, and infecting hypha takes place without any food other than that stored in the spore. The first attempt of the fungus to establish food relations with its host is quite uniformly fatal to the host cell and to the haustorium within it. This wastes the slender resources of the young fungus, and occasionally it dies at this stage. In older material it is not uncommon to find minute dead infections consisting of an empty appressorium and substomatal vesicle at a dead stoma and one empty hypha leading to a single dead host cell.

Commonly, however, the fungus survives and forms successive haustoria in several cells. It may die at any time in this process, or continue a feeble existence for a longer period.

Plate 1, D, depicts the tissues in and around an eight-day infection. The fungus entered through the stoma in an adjoining section, and apparently has been dead for several days. One or two empty, swollen, transparent hyphae remain, as at *a*. Three or four host cells have been killed, one of which is shown at *b*. There is remarkably little disturbance in the surrounding tissues. Cell walls in actual contact with a dead cell, as at *c*, *d*, and *e*, may become greatly swollen, showing several lamellae, and the near-by contents of those cells may

EXPLANATORY LEGEND FOR PLATE 2

A to E.—Details from an 11-day infection, showing dead haustoria. Part of this mycelium is still living ×1130

A.—Haustoria are at *a* and *d*, a living nucleus is at *c*, and dead nucleus at *b*
B.—There is a small dead haustorium, *b*, accompanied by dead nucleus, *c*
C.—Haustoria at *a* and *c* surrounded by living cytoplasm of host cell. Dead nucleus at *b*
D.—Larger dead haustorium, *a*, accompanied by nucleus *b*
E.—Haustorium, *d*, in dead host cell. Disintegrated plastids at *a*, *b*, and *c*
F.—Hypha containing three nuclei, *a*, *b*, and *c*, with a coarse chromatin net and no nucleolus. ×1130
G.—Fungous nucleus, *a*, with both nucleolus and coarse chromatin net
H.—Hypha with two ordinary nuclei, *a* and *c*, and one nucleus, *b*, with heavier net and no nucleolus

be slightly damaged. Beyond this, the tissues look healthy. The entire affected area is microscopic in size and would not be visible to the unaided eye in living material.

If the fungus still is living eight days after inoculation, a much larger area may be involved. The diameter of the mycelium ranges from 100μ to 300μ. Even in the most vigorous mycelium, however, the growth is meager. Plate 1, E, is representative. The fungus entered at *a*, and the guard cells at the point of entry are dead. The single infecting hypha forked at *b*, the branches leading off into the tissues. Not even at the center of the infection are the intercellular spaces in the leaf filled with hyphae. All of the older mycelium is drained and empty, and some of the hyphae are wrinkled and misshapen. Only the marginal hyphae, as at *i* and *j*, are living, and they are not vigorous.

Haustoria are formed but often remain small and dense and die without expanding. A few expand partly, as at *c* and *f*. The average size of the five largest haustoria found was 16.4μ by 4.6μ, which is little over one-third the size of the largest in Little Club (45μ).

The host cells in which haustoria form are killed. The first cells invaded undergo a quick collapse and the cell contents become homogeneous and stain a dense red.

As has been noted in other rust studies (*2, 3*), however, later attacks upon a resistant host, in any given infection, produce less violent results in the host cell than do the earlier attacks. But under these conditions, Malakoff and this form of leaf rust are so highly uncongenial that, even in an older infection, the life of an invaded cell is short. In the infection shown in Plate 1, E, all the cells containing haustoria were counted. About half of them were dead, and nearly all the rest showed marked signs of disorganization. The host nucleus underwent little or no expansion and soon died (pl. 1, E, *g*), and the plastids and cytoplasm diminished and disappeared, leaving the cell clear, colorless, and empty (pl. 1, E, *d* and *e*).

The effect of the fungus on host-cell walls at this stage is practically limited to the invaded cells. Occasionally the wall of an adjoining living cell is affected beyond the limits of its contact with the dead cell, as at Plate 1, E, *h*, but even this is not the rule. A close examination of these swollen walls shows that the outermost layers of wall are first affected. Often the inner layers, those nearest the protoplast, still take normal stain.

The damage done by the fungus at this time (eight days after inoculation) is virtually limited to the infected area and a few cells just beyond its edge. Plastids in newly invaded cells average 3.1μ by 1.8μ in size, those just beyond the marginal hyphae 3.9μ by 2.5μ, and the normal plastids 5.3μ by 2.7μ. No excess of starch has been observed either within or around the infection.

During the next few days there is a marked change in the appearance of both the fungus and its host. In 11-day infections a few of the mycelia are still living although they have increased but little in diameter. Within the infection both host and fungus show profound disturbances. The hyphae are impoverished in content and struggling for existence. Much of the mycelium is drained, and the empty hyphae are swollen and sometimes misshapen. Even in the growing tips one finds more or less vacuolated cytoplasm.

The growth is meager within the infected area, and the fungus is making but feeble attempts to spread to fresh tissues beyond. Stolons are virtually absent.

Nuclei of the fungus often are hard to see and in some cases have undergone a change of structure. The regular fungous nucleus (pl. 1, A, B, and C) consists of a nuclear membrane, a central spherical body, probably nucleolar in nature, and a fine, scarcely visible chromatin network. Some of the nuclei shown in Plate 2 still have this structure (pl. 2, H, *a*, *c*). In other nuclei, as in those shown in Plate 2, F, *a*, *b*, and *c*, and in Plate 2, H, *b*, the nucleolus has disappeared, and the chromatin network has become relatively coarse and heavy. Plate 2, G, at *a* apparently shows a transition stage. This change of nuclear structure was noted in some cases in eight-day infections but is much commoner in older mycelia.

When such a change of nuclear structure was first noted, it was considered possible that one or the other type of nuclear structure was the result of poor fixation. This may be so, but when one finds both types common in the same preparation, and even in the same cell (pl. 2, H), the correctness of such an interpretation becomes doubtful.

A similar alteration in the structure of the fungous nucleus takes place regularly in haustoria, even on susceptible hosts. The single haustorial nucleus contains a coarse chromatin net but no nucleolus. Perhaps this change is of a degenerative nature and occurs when the nucleus is no longer able to divide. Or it may be due to some adverse effect of the host, limited to the haustoria when the fungus is grown on susceptible hosts but tending to spread out into the mycelium when the host is more uncongenial.

In infections of this rust on Little Club (*4*), it was found that the rust nuclei are commonly in groups of three. Even on Malakoff, on which the fungus grows poorly and abnormally, groups of three nuclei are noted (pl. 2, F and H).

As in the younger mycelia, haustoria form, but nearly all soon undergo degenerative changes and die. The cytoplasm and nucleus of the haustorium disintegrate and form a nearly homogeneous substance that stains intensely with safranin. In shape the dead haustorium is usually shorter and thicker than the living haustorium. In some cases no structure can be discerned within the haustorium (pl. 2, D, *a*, and pl. 2, B, *b*). Other haustoria, as those shown in Plate 2, C, *a*, and Plate 2, A, *d*, seem to be composed of concentric layers with differing affinity for stain. Sometimes these haustoria are closely surrounded by cytoplasm and plastids of the host (pl. 2, C, *a* and *c*), while at other times they are fairly free (pl. 2, A, *d*). It is not certain whether the heavy coating of the haustorium in Plate 2, E, *d*, is derived from the haustorium, or the host, or both. The degree of this degeneration of haustoria varies with the vigor of the fungus.

The host nucleus may survive for a short period after the entrance of the fungus into the cell (pl. 2, A, *c*) but usually dies almost immediately (pl. 2, B, *c*). Whether living or dead, the host nucleus is to be found close to the haustorium. The plastids persist for a time, but ultimately disintegrate into red-staining smears (pl. 2, E, *a*, *b*, *c*). Many of these host cells collapse into irregular red-staining masses within which the outline of the haustorium may still be discerned.

The most striking changes 11 days after inoculation, however, are in the tissues beyond the fungus. Plate 3, A, represents a narrow

strip through the leaf from one epidermis to the other, at a distance of 150μ beyond the edge of the mycelium. The cells are living and are in fair condition, but the walls are irregularly swollen. Isolated spots have thickened into little wartlike prominences (*b* and *c*) which stain almost black in the triple stain. Occasional wider areas of wall, as at *a*, *d*, or *e*, fill in a small intercellular space or form a wedge-shaped mass between two lobes of a cell. These swellings occur only in the proximity of the infections in a conspicuous zone, which varies in width from a few microns to 300μ.

The disturbance obviously is due directly or indirectly to the fungus. It is noteworthy, however, that, although each infection is surrounded by a zone with swollen walls, the tissue in the infection itself has little or none of this swelling. (Compare pl. 3, A, with pl. 2, A to H.)

Measurements were made of nuclei and plastids in and around one of these infections. The nuclei of the outer zone show no abnormal expansion in size, the average being 9.5μ by 7.3μ, as compared with 8.9μ by 7.9μ in the healthy tissues beyond. There is remarkably little decrease in cytoplasm and plastids. Plastids in living invaded cells averaged 2.7μ by 1.8μ; just beyond the marginal hyphae they averaged 2.8μ by 1.8μ; 100μ beyond the fungus they averaged 4.2μ by 1.9μ, and the size of plastids in healthy tissue in this leaf was 4.3μ by 2.1μ. Thus it would appear that the partial dissolution of host-cell walls extends much farther beyond the fungus than does the impoverishment of the cell contents, for the cell contents look normal 100μ beyond the fungus, whereas swollen walls may occur for a distance of 300μ. No excess starch was found in plastids of tissues affected by the fungus.

A week later (18 days after inoculation) the effects had become more marked. Plate 3, B, represents a strip through the leaf at the center of the infection, and Plate 3, C, is drawn from the zone of tissue surrounding the same infection, at some distance beyond the outermost hyphae. The leaf tissues of both are dead. At the center, a few remnants of hyphae are still visible. Some of them, as those in Plate 3, B, *a*, still show small amounts of cytoplasm. The mesophyll cells have collapsed and are misshapen, and even the epidermis has flattened out.

In the outer zone (pl. 3, C), which sometimes is 300μ or even more in width, the cell walls usually have not collapsed, but the contents are shrunken and dead, and the walls are thickly covered with coarse black-stained "warts," many times the number and size of those found in the 11-day material. This zone with swollen walls is conspicuous even under the low-power lens of the microscope.

As has been noted already, the swellings on the walls extend outward into intercellular spaces or form between adjoining cells at contact surfaces. This suggests that it is the first-formed layers of wall (the middle lamella and to some extent the secondary lamellae

Puccinia triticina physiologic form 11 on Malakoff wheat. A, B, and C, infections on seedlings grown in bright dry weather, in April and May, 1924; D and E, infections on older plants grown in dark wet weather, in February, 1925

For explanatory legend see page 702
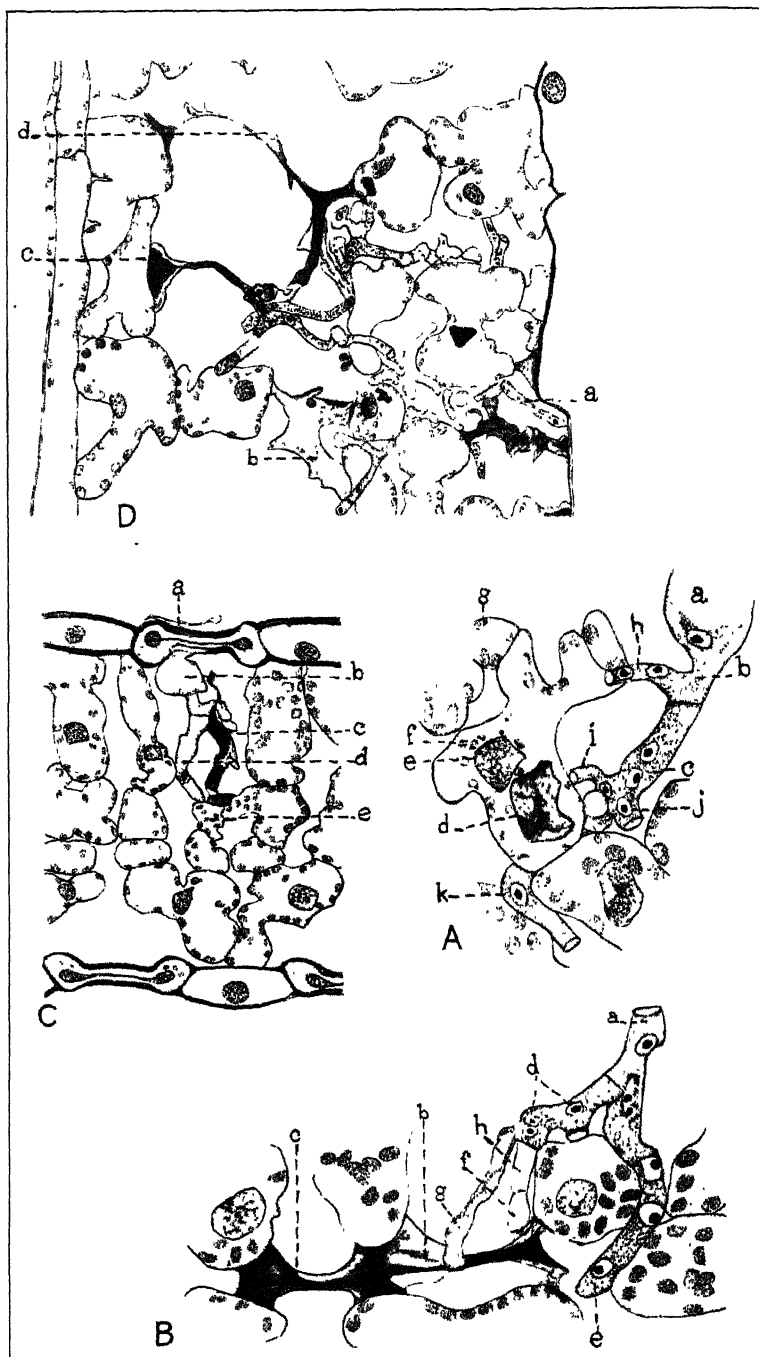
Puccinia triticina physiologic form 11 on Malakoff wheat. Infections on older plants grown in dark wet winter weather, in January to March, 1925

For explanatory legend see page 703

next to it) that are being decomposed. If so, these swellings should contain pectic compounds (*12, 15, 1*) and take the stains for pectose and its decomposition products. The inner, later layers of wall appear to be untouched.

When sections of this material were stained with methylene blue, washed, and mounted in water, the swellings varied from violet to deep purple. This is a typical pectic reaction. Sections were stained lightly with ruthenium red, dehydrated, and mounted in balsam. If the staining is done properly, the cell contents are colorless and the wall swellings are a clear red. This also indicates the presence of pectic materials. Also, it explains the bulkiness of these decomposing walls, for the ability of pectin to swell and gelatinize is well known. With both stains, the most completely decomposed wall substances, i. e., the swellings formed earliest, took little or no color.

An attempt was made to duplicate the condition of these walls artificially. Sections of wheat leaf were subjected to partial maceration in a mixture of absolute alcohol and hydrochloric acid and then washed and stained. Thickened middle lamellae were noted, with larger swellings at the edges, but no typical "warts" were found. Mangin, however, describes an experiment in which were produced results apparently resembling the work of the fungus in Malakoff infections. He says (*12, p. 297*):

La surface externe de la membrane qui limite les méats présente souvent des sculptures formées par des pointes ou des boutons de pectates insolubles, dispersés sans ordre à la surface ou, le plus souvent, fixés sur les bords des cadres d'union. * * * Enfin, dans un certain nombre de cas, les méats sont partiellement ou complètement remplis d'une sorte de gelée due à la transformation de l'acide pectique normalement insoluble, en une masse capable de se gonfler et de se dissoudre dans l'eau. * * *

Ordinarily, the rust dies without attaining even the beginnings of reproductive activity. Only rarely does one find even a minute uredinium. Host tissues die and fungous growth stops after the formation of a few spores. The epidermis of the host may not even be ruptured above the spores.

Infections on older hosts were studied during the following winter. The seed was sown June 1, 1924, in the field, and the plants grew slowly through the summer and fall, forming a heavy rosette. By January 14, 1925, when the first inoculations were made, the plants were pushing up culms and stood 2 feet high. Later leaves on these plants were inoculated a month later (February 19). Material from both lots was fixed at stated intervals after inoculation.

The fungus developed slowly under winter conditions. A few minute, bright-yellow flecks had appeared by the tenth day. These increased slowly in number and size, only a few exceeding a fourth of a millimeter in diameter. No hint of spore production was noted.

For unknown reasons, perhaps associated with winter conditions, the hyphae of a considerable percentage of the appressoria in this

EXPLANATORY LEGEND FOR PLATE 4

A.—Detail of five-day infection showing first attack. The substomatal vesicle is at *a*, the infecting hypha, at *b c*, the first haustorium (dead) at *d*, attended by the host nucleus, *e*. Plastids of the host cell, *f*, are minute. The infecting hypha has branched at *h, i, j, k*. ×730

B.—Slightly later stage. Infecting hypha, *a, f*. The host cell, *b c*, is dead. The haustorium mother cell *f*, and hypha, *h*, are empty. The branch, *g*, is enfeebled. More vigorous branch at *e*. ×730

C.—Portion of leaf section six days after inoculation showing collapsed appressorium, *a*, vesicle, *b*, hypha, *d*, dead host cell, *c*. The fungus is nearly dead. ×333

D.—Seven-day infection that is gaining ground. There are dead host cells at *b, c*, and *d*, and fairly vigorous hypha at *a*. Surrounding tissues show little disturbance. ×333

material failed to enter the stomata, but when entry took place it resembled closely what has already been described. In Plate 3, D, the appressorium, *a*, has formed on the guard cell, *b*, and its contents are moving through the stomatal slit to form the substomatal vesicle, *c*.

Plate 3, E_1 and E_2, recalls at once the cases of fusion of appressoria seen in material of this rust growing on Little Club (*4*). Successive sections of the same stoma are shown. At the center of the appressorial mass (pl. 3, E_1), it appears as a two-lobed figure, *a* and *b*, somewhat constricted at the center. At either side of the center, as in Plate 3, E_2, it appears as two separate masses, *a* and *b*. A single substomatal vesicle, *c*, is forming inside. Appearances suggest strongly that two appressoria fused and are entering as one.

From the rounded vesicle just inside the stoma a single multinucleate infecting hypha pushes out and soon forms contact with mesophyll tissue. As before, the first cell attacked is killed. Plate 4, A, drawn from a five-day infection, shows a step in this process. From the partly evacuated vesicle, *a*, proceeds the hypha, *b c*. It has formed a haustorium, *d*, already dead and partly disorganized. The host cell in which it formed is living but shows marked disturbance. The nucleus and part of the cytoplasm have moved to the haustorium. The plastids, *f*, are reduced in size almost to extinction but, oddly enough, contain a minute amount of starch, in fact more than the healthy plastids of surrounding cells, *g*. Excess starch is fairly common under some conditions in infections on other hosts, but it is rarely observed on Malakoff. The fungus in this case is still alive, and the infecting hypha has produced three or four branches (pl. 4, A, *h, i, j, k*).

In Plate 4, B, also from a five-day infection, is shown a more advanced stage of development. The hypha grew from *a* to *f* and formed a haustorium in the cell, *b c*. This host cell is dead and has collapsed into a stringy mass. Moreover, the haustorium mother cell at *f* has died and nearly disappeared. Apparently the fungous cytoplasm below this cell retreated and formed a septum near *h*. A branch, *g*, has formed near this septum but is in feeble condition. Farther back, a more vigorous branch, *e*, is growing out.

It is noteworthy that often, even under conditions so unfavorable to the fungus that it dies, there is a marked tendency toward the trinucleate condition. Plate 4, A and B, and Plate 5, A, show a number of fungous cells with three nuclei each.

The growth of the fungus is slow and very variable. In Plate 4, C, is shown part of a longitudinal section of the leaf containing a six-day infection. A withered appressorium at *a*, an empty vesicle at *b*, one or two empty hyphae, *d*, and a dead and dried host cell at *c*, and that is all. One other hypha is found in the next section. The fungus is practically dead. Except for a slight reduction in plastid size as at *e*, the adjoining cells are normal.

As was the case when the seedlings were infected, a considerable percentage of the fungi died during the first week. In the later fixations were found minute dead infections consisting, like the one in Plate 4, C, of an empty substomatal vesicle, one or two empty and partly disintegrated hyphae, and, associated with them, one or two collapsed host cells. Sometimes only traces of the former hyphae remained.

If, however, the fungus remains alive through the first week, it stands a fair chance of living much longer, for a process of adjustment takes place here similar to that described above in connection with Malakoff seedlings and other rusts (*2, 3*). Invaded host cells live a little longer, and haustoria expand partly and extract some food for further growth before the host cell dies.

The infection shown in Plate 4, D, is 7 days old and is making progress. The mycelium extends through several sections. It is a sparse growth, but there are several living hyphae, and the fungus is slowly gaining strength. The host cells die soon after being invaded (*b, c, d*) but not until the fungus has extracted enough food to enable it to grow to the next cell. No gelatinization of host-cell walls either within or beyond the infection was noted at this stage, and the impoverishment of tissues beyond the infection was negligible.

A week later, in 14-day infections, the fungus had not increased greatly in diameter. A few mycelia were measured and were found to range from 120μ to 300μ in diameter. In the one shown in Plate 5, B, the fungus was still alive, but some hyphae were dead (*h*), and others were empty or thin in content. Many of the haustoria fail to expand but undergo the type of degeneration seen so commonly in the seedling material. An increasing number, however, expand partly and function for a while. The average of the largest is 34μ, whereas the maximum on a congenial host is close to 50μ.

The damage done to the host is localized within the minute area occupied by the fungus. In this area, cells are dead or dying. The cells that were first attacked have collapsed (*e* and *j*). Within the smeared-looking, red-stained contents of these cells one sometimes sees remnants of nucleus and haustorium (*j*). Not all of the cells entered later by the fungus collapse in this fashion. Instead, the nucleus dies, the plastids dissolve (pl. 5, B, *k, i*), and the cell may become clear and empty (*f*). This is a slower and apparently less extreme reaction to the fungus and is found only in older infections. Occasionally a wall in contact with a dead cell (*a* and *d*) will swell up, showing the lamellae of which it is composed.

The impoverishment of tissues surrounding the infection is slight. Two or three cells beyond the fungus (pl. 5, B, at top of drawing) the tissues are nearly normal. The plastids are slightly decreased in size and the nuclei are somewhat expanded, measuring 10μ by 8.8μ as compared with the normal size of 9μ by 7.6μ.

The cell walls in this outer zone are virtually unchanged, although here and there one finds minute swellings (*c, l*) of the sort so abundant near the seedling infections.

The oldest infections studied on these plants were 19 days of age. The rust had been growing faster and at this time the infections ranged from 150μ to 750μ in diameter. This increase took place by the slow spreading of feeding hyphae, no runners having been noted.

At the center of the larger 19-day infections both host and fungus are dead. That shown in Plate 5, C, is typical. The host cells have collapsed and their broad lobes have shriveled into strands (*b, c*). Within these cells occasional remnants of small spherical haustoria, *a*, are still visible. Hyphae have increased in number. They are dead and empty but still persist. There seems to be little disappearance of mycelium here.

Out toward the margin of this same infection, intermingled with collapsed cells, are a few which have retained their original shape (pl. 5, D). They are deficient in contents but not plasmolyzed. The nucleus usually is dead (pl. 5, D, *b*, *c*), and the plastids are minute. In appearance they resemble fairly closely the corresponding tissues in an old infection on a susceptible host. The development of the haustorium, too, had run a nearly normal course; it had expanded partly (it was 25µ long), had functioned without causing collapse of the host cell, and had undergone normal drainage, leaving it empty and clear.

In the beginning, haustoria remained as minute dense bodies which died without expansion. Some gradual change in the relation of host and parasite made possible greater expansion and more normal functioning of haustoria and, correlated with it, an increased rate of growth of the hyphae. At its best, however, the fungus makes but a feeble advance, and even in old infections, some host cells die soon after being entered. In Plate 5, E, at the very edge of the infection, are two collapsed cells, *a* and *b*.

Swellings on host-cell walls are still few and occur more commonly just beyond the infection than in it. The cells in Plate 5, D, have unmodified walls, although they are surrounded and invaded by the fungus, whereas the uninvaded cells in Plate 5, E, *c*, *d*, and *e*, bear little hemispherical swellings. Warts do occur on walls of attacked cells (pl. 5, E, *a* and *b*) but may have formed before the fungus reached them. Certainly the great majority of warts are found on cells not in direct touch with the fungus. The disintegration of host-cell walls is a minor factor in this material.

On the whole, the development of the organism is sharply restricted. In many instances the organisms die during the first week. The few that survive gain slowly. The host tissues at the center of the mycelium die, but in the further spread of the fungus some sort of tolerance is developed that allows haustoria to expand more and take more food and the hyphae to grow faster. The average diameter of the 19-day mycelia is 429µ, nearly twice that of the seedling infections of the same age. Although the vegetative growth of the fungus is greater than that in the seedling infections, no trace of reproductive activity has been found here.

On comparing the infections on seedlings grown in the spring with those on older plants in the winter, differences are noted. Old infections on seedlings are small and are surrounded by a broad zone of tissue whose walls undergo partial disintegration and whose contents die. Infections of the same age on grown plants are larger. They have but a small trace of this disintegration of walls, and the tissues around the infection are living. It is not clear whether these differences are due to the difference in the age of the hosts or to differences in weather conditions.

Puccinia triticina physiologic form 11 on Malakoff wheat. Infections on older plants inoculated during dark wet weather, in January and February, 1925

For explanatory legend see page 706
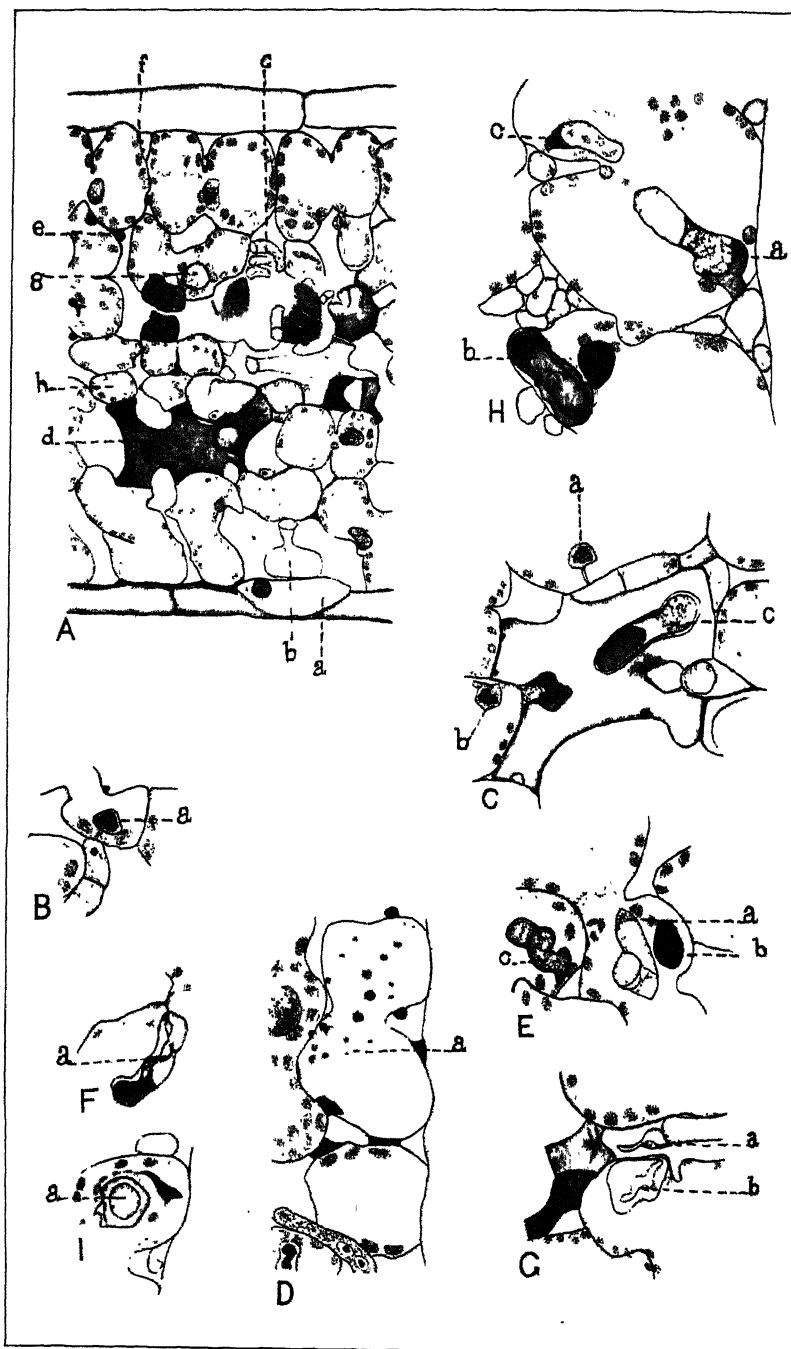
Puccinia triticina physiologic form 11 on Malakoff wheat Infections on seedlings inoculated during dark wet weather, in January, 1925

For explanatory legend see page 707

Infections on a third set of plants served as a check. The seed was sown beside the older plants January 17, 1925. The seedlings were inoculated on February 26, and encountered the same dark wet weather as the infections on the older plants.

Here, as before, macroscopic evidence of infection was slow in appearing. Even on the fourteenth day the flecks were few, minute, and vague. On the eighteenth day they had turned bright yellow, but even the largest was less than a millimeter in diameter.

Microscopic study shows that the fungus experiences the same severe struggle for existence during its early life that was seen in the other material. Even on the ninth day the fungus was not well established in the host. Plate 6, A, represents the central section through a nine-day infection. The fungus entered through the stoma of which a is an accessory cell. The empty substomatal vesicle, b, and a few empty hyphae, such as those at c, make up the sparse growth of the fungus at the center. Marginal hyphae in adjoining sections look more vigorous, but there are only a few of them. Host cells collapse very soon after they are entered (pl. 6, A, d). Young haustoria are drawn in Plate 6, B, at a, and Plate 6, C, at a and b. Haustoria may expand partly (pl. 6, C, c) but, as in this case, are soon checked by the dissolution of the host cell.

The disturbance caused by the fungus extends but little beyond the infection itself. Near-by host nuclei are enlarged somewhat (pl. 6, A, g), plastids may be reduced (pl. 6, A, h), and an occasional cell wall bears one or two wartlike swellings (pl. 6, A, e and f). An exceptional cell at the edge of the mycelium (pl. 6, D, a) bears numerous little swellings. The cell is living, but the contents have been omitted for clearness. In general, wall disturbances were slight and extended only two or three cells beyond the fungus.

On the fourteenth day the fungus was in somewhat better condition. The largest living haustoria averaged 15μ in length, but they often were twisted and irregular (pl. 6, E, a and c) and soon showed signs of degeneration (pl. 6, F, a). The final condition is shown in Plate 6, G, b, where the haustorium has changed into a short, irregular lump, taking little or no stain, and within which a vague concentric stratification can be discerned. The haustorium mother cell outside (pl. 6, G, a) is crumpled and shrunken. The host cell containing this haustorium has not collapsed.

On the fourteenth day a narrow zone around the infection showed slight impoverishment. There was very little breakdown of wall materials.

In the 18-day material about 20 living infections were studied. Mycelia had attained greater size in this than in any other Malakoff material studied. The largest mycelium was nearly a millimeter in

EXPLANATORY LEGEND FOR PLATE 6

A.—Center of a nine-day infection. The accessory cell of the stoma of entry is at a, the substomatal vesicle, at b, a few drained hyphae, at c, a dead host cell at d, swellings on the walls, at e and f, and expanded host nucleus, at g. $\times$333

B.—Detail of nine-day infection, showing young haustorium at a. $\times$730

C.—Half-grown haustorium, c, checked in its development by death of host cell. Young haustoria at a and b $\times$730

D.—Cell at edge of nine-day infection, covered with numerous minute wartlike swellings of outer pectic wall layers. $\times$730

E.—Haustoria, a and c, somewhat twisted and contorted. Dead nucleus at b. $\times$730

F.—Haustorium in early stage of degeneration. $\times$730

G —Detail from 14-day mycelium. Crumpled haustorium mother cell at a. Degenerated haustorium, showing concentric stratification at b. $\times$730

H.—An 18-day infection, showing stages of degeneration of haustoria at a, b, and c. $\times$730

I.—Dead haustorium from 18-day infection. $\times$730

diameter and the average was about 660μ. Much of this increase had come in the last few days, and on the whole there was an appearance of greater congeniality than there had been earlier.

The fungus had made relatively rapid growth, but with few exceptions the mycelium was not dense. The hyphae had normal diameters, but the cytoplasm was vacuolated. In general, only the growing tips of the hyphae had dense contents. Probably 95 per cent of the hyphae were drained and empty. Haustoria formed and expanded to greater size than in the younger infections. The average of the 10 largest found in one infection was 24.1μ. This, to be sure, is but little over half the size attained by haustoria in a susceptible host, but it is much greater than was found in younger infections in this material. Nor does this mean merely that haustoria take all this time to grow up. The earlier-formed haustoria were dead by this time. These haustoria were closer to the margin of the mycelium and were of later growth. Some of these haustoria (pl. 7, B, a) had a normal appearance and underwent normal drainage. The host cell was still vigorous, and its contents were normally disposed.

But here, too, degeneration slowly sets in. The neck and the basal end of the haustorium break down into matter that stains dull blue (pl. 6, H, a). Gradually more and more of the haustorium becomes involved, the change sometimes progressing faster along the surface than in the interior. Finally the whole haustorium becomes changed to a blue-staining mass consisting of an outer layer and an apparently homogeneous interior (pl. 6, H, b, and I, a, and pl. 7, C). It is still uncertain whether this outer layer was derived from host, or fungus, or both.

In tissues back nearer the center of the infection, that have been exposed longer to the fungus and are nearly spent, the fungus may still attempt to form haustoria. The hyphae contain little cytoplasm, but it is enough to form a haustorium mother cell. In Plate 7, D, there is an old dead haustorium at a and the beginning of a new one at b. The contents of the haustorium mother cell at c have only begun to enter the host cell, but already the mother cell has been altered chemically (it stains bright blue) by some substance that has diffused outward from the host cell. These blue-staining mother cells are of frequent occurrence in this area.

Even here where the fungus attains its greatest vegetative activity, the majority of the mycelia show no hint of reproductive activity. Occasionally there is a slight massing of hyphae below the epidermis. In only one infection (pl. 7, A) was a small uredinium (450μ in diameter) formed. Even in that instance the first spores died without maturing, but later spores looked more normal.

As may be seen from the drawing (pl. 7, A), nuclear phenomena in connection with reproduction are fairly regular. Spores, stalk cells, and some of the cells beneath are usually binucleate. There are exceptions. Plate 7, E, for instance, shows a stalk cell with three

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EXPLANATORY LEGEND FOR PLATE 7

A.—Section through a small uredinium. Spores and stalk cells binucleate. Majority of host cells not collapsed. ×333

B.—Detail of 18-day mycelium, showing haustorium of normal appearance. ×730

C.—Dead haustorium with sheath. ×730

D.—Detail near center of 18-day infection. Old dead haustorium at a from collapsed mother cell at d. Beginning of a new haustorium at b. Blue-stained haustorium mother cell at c. ×730

E.—Detail from uredinium, showing a stalk cell with three nuclei, b, c, d, and a doubtful fourth nucleus at e, and a spore mother cell with one nucleus, f, and a second, a, containing two nucleoles. ×730

F.—Marginal region of 18-day infection. The outermost hyphae are at a, b, c, small haustoria at d and f, and slight swellings of walls at g, h, and i. The host tissues are living. ×333
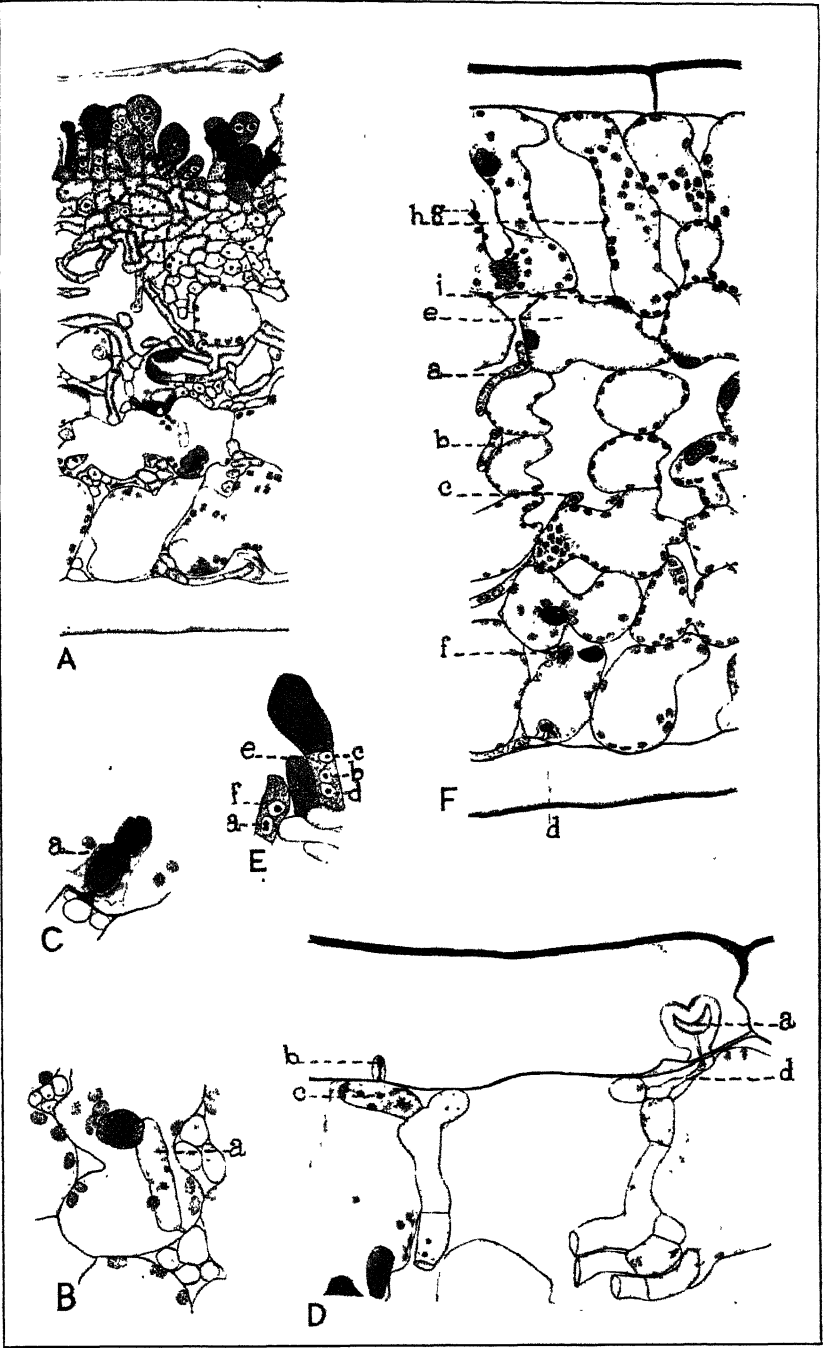
Puccinia triticina physiologic form 11 on Malakoff wheat.   Infections on seedlings inoculated during dark wet weather, in January, 1925

For explanatory legend see page 708

good nuclei, *b*, *c*, and *d*, and a doubtful fourth nucleus at *e*. In the same figure is a spore mother cell with one ordinary nucleus (*f*) and a second (*a*) which looks double and contains two distinct nucleoli.

Out in the mycelium there are more frequent irregularities in nuclear content. There are more binucleate cells in Malakoff than in Little Club. Cells of the hyphae contain anywhere from two to eight nuclei, and the haustorium mother cells may have two, three, or four. This breakdown in the regularity in the number of nuclei probably is associated with the adverse conditions under which the fungus lives.

In the later-invaded areas, the host, too, shows evidence of somewhat greater congeniality. The damage done during the early period still persists, of course, at the center of the infection, but as the fungus spreads outward to fresh tissues the host cells show progressively increasing tolerance. In the earlier reaction as the cell collapses the cell contents break down rapidly into a homogeneous substance that stains intensely with safranin. That occurs but rarely later on. In all the marginal regions of the fungus, host cells maintain their original size and form. The majority of invaded cells are still living, although the plastids tend slowly to decrease in size, the nuclei die prematurely, and in the end the cells become clear and empty.

Apparently the shift toward susceptibility is not great enough to bring into play the heightened cell activities seen on susceptible hosts. The normal nuclei of healthy tissue average 9.6μ in diameter, the living nuclei in cells bearing haustoria average 8.6μ, and the dead nuclei average 6.1μ. So far as can be judged by this, the nuclei have not expanded but have undergone slow contraction and death. The plastids decrease in size, and at no time has excess starch been seen in them. These cells, however, do show higher osmotic pressure than does healthy tissue.

The area immediately surrounding the fungus (pl. 7, F) shows almost no disturbance. The outermost hyphae are at *a*, *b*, and *c*, and there is a small haustorium at *d*. The cell at *e* has diminished protoplasm, and the one at *f* a dead nucleus. At *g*, *h*, and *i* are localized swellings of the pectic layers of the walls. Apart from that, the tissues appear healthy. This is in striking contrast to the appearance of the outer zone in the 18-day infections on the first set of seedlings studied (pl. 3, C), the tissues of which were dead and the walls heavily coated with large wartlike swellings.

In order to bring together the facts bearing on this point, the data have been tabulated (Table 1). The first set of seedlings had strong light, no rain, the minimum of watering, a mean humidity of 72, and an average temperature range of 50° to 65° F. Under these conditions the mycelium spread little, the average extent at 18 days being 235μ. A few infections produced minute uredinia, but each infection was surrounded by a broad zone of tissue in which the walls showed pronounced disintegration, and the contents were dead by the 18th day. The second set of seedlings and the older plants during the period following inoculation encountered short days and dark weather, over 11 inches of rain, a mean humidity of 79, and an average temperature range of 49° to 60° F. On the older plants the infections had nearly twice the diameter (429μ) of those of the first seedlings but bore no spores. The diameter of the infections on the second set of seedlings (661μ) was nearly three times that on the

first lot. In both, the effect upon the walls in the area surrounding the infection was slight, and the tissues were still living 18 days after inoculation. It would appear, then, that weather conditions have a greater effect than the age of the host in producing the differences noted.

TABLE 1.—*Dates of operations, summary of the weather conditions, and their effects on three lots of infections by Puccinia triticina physiologic form 11 on Malakoff wheat*

DATES OF OPERATIONS

Item	First seedlings	Second seedlings	Older plants
Date of planting	Apr. 23, 1924	Jan. 17, 1925	June 1, 1924.
Date of inoculation	May 4, 1924	Feb 26, 1925	Jan. 14 and Feb. 19, 1925.
Date of last fixation	May 22, 1924	Mar. 16, 1925	Mar. 16, 1925.

SUMMARY OF WEATHER CONDITIONS

	First seedlings	Second seedlings	Older plants
Rainfall	0 inches during the experiment. The plants were watered only enough to prevent wilting. (Rainfall below normal in preceding winter.)	From Jan. 14 to Mar 16 11.57 inches (heaviest rainfall between Feb. 3 and Feb 23).	From Jan. 14 to Mar. 16 11.57 inches.
Temperature.[1]			
Average of daily maxima	65° F.	60° F.	60° F.
Average of daily minima	50° F.	49° F.	49° F.
Percentage of possible sunshine[1]	April, 73 per cent. May, 69 per cent	January, 73 per cent. February, 55 per cent. March, 79 per cent	January, 73 per cent. February, 55 per cent. March, 79 per cent.
Mean humidity[1]	April, 70. May, 74	January, 82. February, 80. March, 74	January, 82. February, 80. March, 74.

EFFECT ON INFECTIONS

	First seedlings	Second seedlings	Older plants
Average diameter of living 18 or 19 day mycelia	235μ	661μ	429μ.
Disintegration of pectic wall materials in zone surrounding infection	Pronounced	Slight	Slight.
Spore formation	Very little	Very little	None.

[1] San Francisco reports.

DISCUSSION

Malakoff wheat is highly resistant to *Puccinia triticina* physiologic form 11 at all ages and under all environmental conditions tested by the writer. Under the microscope, however, there are differences in the appearance of the infections developed under different weather conditions. Chief among these is the difference in the pectose reaction, for in one set of infections the swelling and gelatinization of pectic wall materials is pronounced; in the other two, it is relatively slight.

At least nine-tenths of the decomposition of host-cell walls occurs in cells beyond the mycelium. The fact that a cell attacked by the fungus bears "warts" does not prove that these swellings did not form before the fungus reached it. It seems safe to infer, however, that this breakdown of pectic compounds is due directly or indirectly to the fungus, as it occurs only in the neighborhood of infections.

It is conceivable that acids formed during the breakdown and death of host cells at the center of the infection might be capable of producing such an effect, but, on such a supposition, it is not clear why they do not produce greater effects on the cells within the infection than on those farther out.

This breakdown of pectic compounds more probably is a reaction to an enzyme. Many parastic fungi and bacteria produce pectinase, an enzyme which diffuses out ahead of the fungus ("acts in advance"), breaking down the middle lamellae of the host-cell walls and causing maceration of the host tissues.

Harter and Weimer (*9, 10, 11, 16*), in extensive studies on Rhizopus, have shown (*9, p. 623*) that *Rhizopus tritici* Saito "produces a powerful intracellular and extracellular pectinase," capable of macerating disks of raw sweet potato. A comparative study of several species of Rhizopus (*16, p. 132*) showed a marked variation in the amount of enzyme produced. It is almost absent (*11*) in *R. nigricans* Ehr., a parasitic species. The enzyme is produced at any temperature at which the fungus will grow.

If this reaction in the tissues about the infection is due to an enzyme from the fungus, it may be asked why the cells closest to the fungus are not affected most. Carré finds, from work on the pectic constituents of apples, that acids affect the activity of the enzyme, and suggests (*5, p. 837*) "that the enzymes effecting the decomposition of the pectic acid produced from the hydrolysis of the other pectic substances in the tissues can only operate when the acidity of the cell sap is lowered." It may be that within the mycelial area where host cells are dying, the breakdown of protoplasm gives rise to organic acids which check the activity of the enzyme, and that it can affect cell walls only when the enzyme has diffused out beyond the acids.

This reaction took place only in the case of infections on seedlings exposed to bright weather, a temperature range of 50° to 65° F., and a scant water supply. The infections in wet, dark weather, with a temperature range of 49° to 60° F., were nearly free from this decomposition of walls.

According to Dickson, Eckerson, and their associates (*6, 7, 8*), the composition of the cell walls of seedling wheat is influenced profoundly by the temperature of the soil in which it is grown. At high soil temperatures the walls are composed chiefly of pectic substances; at low temperatures the walls are principally cellulose. These writers find that susceptibility to the seedling-blight fungus, *Gibberella saubinetii* Durieu and Mont., is correlated with the character of the cell walls of the host. The fungus makes poor headway against cellulose walls, but penetrates pectic walls readily and apparently uses pectic materials as food. They find further that low soil moisture and low light intensity favor the development of the fungus.

Here, too, under the different weather conditions, there would appear to be a difference in the composition of the cell walls, perhaps due more to the extreme difference in soil moisture than to the slight difference in temperature. Little difference in susceptibility can be ascribed to it in this case, however, as the parasitism in rusts is of a very different type from that of Gibberella. Rust hyphae remain intercellular, and the host cells are entered only by haustoria, which seem to penetrate pectose and cellulose with equal ease. So far as is known, wall materials of the host are not used as food by cereal rusts.

It may be that this enzyme is produced in about equal amounts in all three experiments but that when the walls of the host are composed chiefly of cellulose the enzyme produces little disturbance. Only when, under different environmental conditions, the metabolism

of the host is altered and its cell walls contain more of the pectic compounds does the enzyme produce a conspicuous effect.

There is an alternative possibility. Harter and Weimer (*10*) have shown that in Rhizopus the substrate influences the production of pectinase. In several synthetic media lacking pectin, the enzyme is absent; when pectin is present, the enzyme is produced. It is possible that the production of pectinase is similarly regulated in this case.

A further fact should be considered. In studies of this same physiologic form of leaf rust on Little Club (*4*), a susceptible host, one set of rusted seedlings was grown beside the first set of Malakoff seedlings, inoculated April 30, 1924, and was subjected to the same temperature, bright weather, and scant water supply. There was no trace of decomposition of pectic wall materials in or about the infections on Little Club.

It may be, of course, that the composition of cell walls of Little Club leaves differs from that of Malakoff even when grown under parallel conditions. Dickson and Holbert (*7*) find such differences between varieties of corn. It also may be that the walls are the same and that the fungus contains the same enzyme. Muhleman (*13*) finds that under the conditions of his experiments the mycelium of Sclerotinia produces pectinase but that it does not excrete it into the culture media. Perhaps *Puccinia triticina* physiologic form 11 regularly produces pectinase but does not excrete it. In that case the enzyme would be released only when the fungus is damaged as on a resistant host.

The question is complicated by too many possibilities, and the exact knowledge is too scant to permit any final decision.

In all three lots of infections studied, the initial reaction of the host to the rust is one of extreme intolerance, and in all three such mycelia as survive the critical period of the first few days gain slowly in strength. Some of them live on for two or three weeks. The chemical interplay of fungous secretions and host reactions becomes regulated or altered or adjusted in some fashion so that the rust thrives to a greater degree.

It is not known how widespread this chemical interplay is. It has been observed before in infections of stem rust on Mindum wheat (*2*) and Khapli emmer (*3*).

The nature of this adjustment process is purely a matter of conjecture. The first haustorium evidently secretes some substance into the host cell which kills the cell. It may be that the fungus gradually stops secreting this substance, and so, by permitting the host cells to survive, can itself make better growth. Or, as has been suggested before (*3, p. 722*), the first secretion of this harmful substance may diffuse out slowly into adjoining host cells, setting up there some counter-reaction, perhaps in the nature of antibody formation, by which, when these cells in turn are invaded by haustoria, the harmful fungous secretion is neutralized. The host cell then survives, the rust has living cells from which to draw food, and if its own metabolism is not interfered with by the reactions of the host, a mode of living is established. If this supposition is correct it is, by an odd reversal of what one would expect, the defensive reaction of the host which renders it more susceptible, for this highly specialized parasite lives only when its host is able to survive.

The difference between the initial reaction and the final one is not great here. Graded on the usual scale of 0 to 4, even the best reaction would rank as 0 + or 1, for the few uredinia produced are small and often remain subepidermal. In other material, however, the spread between the initial and final reaction is greater (unpublished data). Some infections remain microscopic in size; others that succeed in surviving the critical stage may bear fair-sized uredinia. It may be that this is one of the factors operative in producing the "X" or "heterogeneous" reaction noted by Stakman and Levine. They define this reaction (*14, p. 5*) as follows: "Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill-defined." This adjustment process may be one of the factors, but it is almost certainly not the only one contributing to the production of the heterogeneous type of infection.

SUMMARY

Malakoff wheat is highly resistant to *Puccinia triticina* physiologic form 11 at all ages of the host and under all environmental conditions tested.

In the young infections the first host cells invaded collapse and die promptly. Many of the young rust fungi die at this stage. If they survive this first severe struggle for existence they gain slowly in strength. As successive host cells are entered by haustoria, the host reaction becomes milder and slower until finally a haustorium may form, expand partly, function for a short period, and undergo normal drainage. The rate of growth of the mycelium becomes correspondingly increased. A few of the mycelia bear small uredinia.

It is suggested that this adjustment process may be one of the factors operative in producing the "X" or "heterogeneous" type of infection described by Stakman and Levine.

In infections exposed to bright weather and scant water supply, a zone of host tissue surrounding the infection shows pronounced degradation of the pectic constituents of the cell walls. In seedlings and older plants inoculated during the dark wet weather of January and February this reaction is slight and sometimes absent.

This breakdown of pectic substances may be due to acids formed in the dying and decomposing host cells within the mycelial area, but it is more probably due to an enzyme from the fungus.

The difference in the amount of this pectic reaction in plants subjected to different environmental conditions may be due to a difference in the composition of the mesophyll cell walls brought about by those conditions.

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THE SWINE HERDBOOK AS A SOURCE OF DATA FOR THE INVESTIGATION OF THE SEX RATIO AND FREQUENCY OF SEX COMBINATIONS IN PIG LITTERS [1]

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INTRODUCTION

The vast number of pigs so far registered by the various breed associations in this country and others would be greatly in excess of the needs for a satisfactory statistical investigation of sex ratio and sex constitutions of litters were it not for the fact that in most cases the data are incomplete. When there is omitted from the record the sex constitution at birth of the litter to which the registered animal belongs, the record becomes worthless for any study in which sex is concerned. Some swine associations do not record the size of the litter, others record the size of the litter and the number of animals raised, while still others record the size of the litter and the sex of those raised. Examples of the last class are the Chester White and the National Duroc-Jersey Associations. But of the millions of litters registered by these two associations only a small percentage can be used for study because only those litters in which all the pigs were raised can be taken. Thus, the data become reduced to a relatively small amount.

Practically all the references to sex ratio and the frequencies of sex combinations in swine are based on herdbook data. Among the citations of sex ratio are those of Crew (*2, p. 255*),[2] who gives 111.8:100 and Rice (*6, p. 158*), quoting Wilkens, who gives 108.74:100. Parkes (*5*) reports 104.7:100. Carmichael and Rice (*1*) observed a ratio of 108.16:100. Wentworth (*7*), studying the frequency of sex combinations, found little divergence from probability. A similar result was obtained by Duncker (*3*). The work of the last two writers is incomplete, however, since only a few litter sizes were studied. A much more extensive study of the situation has been made recently by Parkes (*5*), who used data taken from the National Duroc-Jersey Record. Among 2,020 litters ranging in size from 2 to 14 and comprising 16,233 young he found the proportion of boars to be 0.4884. The frequencies of the sex combinations in the different litter sizes were found to diverge greatly from the calculated frequencies on the basis of the expansion of the binomial $N (p q)^n$, in which p is the mean proportion of boars, q the mean proportion of sows, N the number of litters, and n the size of the litter. Only in litters of five was there a reasonably close agreement between the observed and the calculated frequencies. The exceedingly poor fits seemed in general to be caused by an excess of litters in the mean sex combinations and a deficiency of litters in the extreme sex combinations. Such a condition is, as Parkes indicates, very difficult to explain on biological grounds.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 8
Apr. 15, 1927
Key No. A-117

(715)

MATERIAL

The present study is based on two entirely separate sources of data: (1) The records of the Chester White Swine Association, and (2) the compiled records of the experimental herds maintained by the United States Department of Agriculture at Beltsville, Md., and various field stations. The experimental data were used for the purpose of checking the herdbook data.

In the Department of Agriculture data all litters recorded, except those having one or more pigs of undetermined sex, were included. These data are accurate and for the purposes of the present study include size of litter, number of pigs born alive, sex of pigs taken at birth, and complete information on mortality to weaning at 10 weeks of age. It is thus possible to study sex ratio among stillborns as well as among those born alive and to determine whether there is differential mortality between birth and weaning. The pigs were distributed among the Poland-China, Duroc-Jersey, Tamworth, and Chester White breeds.

The Chester White Swine Record gives the name and number of each animal, date born, size of litter, the sex of those raised, and the names of the parents. The only items used in this study are size and sex constitution of litters. Furthermore, only litters in which all the pigs were raised could be included, since only in such litters was the sex of all pigs born given. The method used was to start at the beginning of volume 26 of the Chester White Swine Record and tabulate the sex constitution of all complete litters of which the registered boars were members, care being taken to avoid duplicate litters. This was continued until 1,541 litters had been tabulated. There was no reason for discontinuing the tabulation at this point except that it was considered to be sufficient.

It should be explained that in the front of the Chester White Swine Record only boars are registered and in the back only sows are registered. Thus the 1,541 litters which were tabulated were recorded because one of each litter was a registered boar. In such data it would be impossible to find a litter consisting of all sows, because in such a case, if the entire litter were registered, they would appear among the sows in the back of the volume. It was necessary to tabulate another 1,541 litters from that part of the record in which the sows are registered, thus doubling the number of litters for study, and giving an equal chance for the occurrence of unisexual litters. Volume 27 was used for obtaining the 1,541 litters from registered sows. The purpose in using a different volume was primarily to avoid taking litters which were duplicates of those taken from the boars.

It was found that the number of litters larger than 12 was very small and for purposes of detailed study only litters of 12 and less were included.

PROPORTION OF BOARS

Consideration will first be given to the proportion of boars found in the Chester White Record data. In Table 1 are shown the number of litters and the number and proportion of boars. The most noticeable feature of these data is the low proportion of boars which, in all litters except 2, 3, and 5, is below 0.5000. The mean proportion comes out $0.4852 \pm .0022$. The mean proportion of boars found

by Parkes (5) in 2,020 litters of 2 to 14 taken from volume 47 of the National Duroc-Jersey Record was 0.4884 ± .0026. The difference between these two results is only 0.0032 ± .0035, a difference which certainly is not significant. Their average is 0.4865 ± .0018.

TABLE 1.—*Size of litters, number of pigs, and number and proportion of males in 3,082 litters from the Chester White Swine Record*

Size of litter	Number of litters	Number of pigs	Number of males	Proportion of males
1	0			
2	14	28	14	0 5000
3	72	216	109	.5046
4	116	464	207	.4461
5	226	1,130	574	.5080
6	360	2,160	1,074	.4972
7	452	3,164	1,543	.4877
8	712	5,696	2,744	.4817
9	472	4,248	2,039	.4800
10	410	4,100	1,989	.4851
11	178	1,958	943	.4816
12	70	840	410	.4881
Totals	3,082	24,004	11,646	.4852

There is reason for believing that the foregoing figures for proportion of boars are too low. Carmichael and Rice (1) report 0.5196 of males in a population composed of several breeds. Severson,[3] in a preliminary report, gives a mean proportion of 0.5230 ± .0056 males in a group of 3,779 pigs representing five pure breeds. The United States Department of Agriculture data, which are given in Table 2, show a mean proportion of 0.5199 ± .0038 males in 7,854 pigs, consisting of several pure breeds. None of these determinations differ statistically from one another. Their average comes out 0.5201 ± .0026 males among 17,290 pigs. The difference between this figure and 0.4865 ± .0018 for the herdbook material is 0.0366 ± .0031. Since this difference is almost 11 times its probable error there can be no doubt about its significance, and it seems probable that the proportion of males shown by the herdbook data is too low by about 3 per cent.

TABLE 2.—*Size of litters, number of pigs, and number and proportion of males in the litters of the Bureau of Animal Industry herd, 1921 to 1926*

Size of litter	Number of litters	Number of pigs	Number of males	Proportion of males
1	7	7	3	0.4286
2	17	34	22	.6471
3	32	96	51	.5313
4	43	172	97	.5640
5	44	220	122	.5545
6	74	444	240	.5405
7	98	686	364	.5306
8	106	848	439	.5177
9	137	1,233	635	.5150
10	107	1,070	555	5187
11	85	935	490	.5241
12	60	720	361	.5014
13	46	598	308	.5151
14	28	392	205	.5230
15	11	165	79	.4788
16	7	112	56	.5000
17	5	85	40	.4706
18	1	18	9	.5000
19	1	19	7	.3684
Totals	909	7,854	4,083	.5199

[3] SEVERSON, A. PROLIFICACY OF SOWS AND MORTALITY OF PIGS. Amer. Soc. Anim. Prod. Proc. 1925/26, (In press.)

The low proportion of males shown by the herdbook probably is to be explained upon the basis of faulty data rather than upon any biological grounds. There are, of course, many ways in which errors could be introduced, either intentionally or unintentionally. A few of these possibilities may be considered briefly. From the very nature of the case, however, the means by which the defects were introduced can only be speculated upon.

Failures of the breeders to report stillborn pigs would affect the sex ratio in case the proportion of males among stillborns differs significantly from the average. Data collected by the Department of Agriculture show that the proportion of boars among the stillborns is $0.5639 \pm .0136$; Carmichael and Rice (1) report 0.5600. These figures are in excess of the proportion of males among those born alive by about 4 per cent and are in line with what would be expected on the basis of Crew's (2) findings relative to the effect of prenatal death on the sex ratio in swine. Crew reports a sex ratio of almost 150:100 at conception. This diminishes to nearly equality at birth and it follows that a much larger proportion of boars than sows is to be expected among prenatal deaths. Whether the omission of all stillborns would be sufficient to lower the proportion of boars to that which has been found to exist in the herdbook depends on the proportion of stillborns which occurs. The number necessary to lower the proportions of males in the Chester White herdbook from an assumed original 0.5320 at birth to the observed 0.4852, assuming a proportion of 0.5600 males among the stillborns, has been calculated and found to be the absurd number 20,894. That is, 44,900 pigs containing 52 per cent of boars at birth and having 20,894 stillborns, among which the proportion of boars was 0.56, would show a proportion of 0.4852 boars if the stillborns were omitted. Such a number of stillborns is 46.5 per cent of the theoretical original population. The percentage of stillborns observed in the Department of Agriculture herd was 7.7. Carmichael and Rice (1) report 9.3 per cent and Severson[4], 3.6 per cent. All these figures are from experimental herds and are not strictly comparable with the herdbook data, but they indicate that probably 7 per cent would be a fair estimate. This suffices to show that anything like the calculated 46.5 per cent is impossible. From the breeder's standpoint also it seems almost impossible that there should be a widespread custom of omitting stillborn pigs from the report filed with the breed association, although it is probably done by some individuals. It seems certain, then, that the omission of stillborns is not the decisive factor in bringing about the low proportion of boars observed in herdbook data.

There is a possibility that the differential mortality among the sexes may influence the proportion of boars observed in herdbook data. According to Crew (2) the male pig is less able than the female to withstand the stress of functioning both before and after birth. If this is true a litter having a high percentage of sow pigs should stand a better chance of being raised in its entirety than a litter having a high percentage of boars. For this reason it is possible that those litters which appear in the herdbook as entire litters have a slightly higher percentage of sow pigs than is representative

[4] SEVERSON A. Op. cit.

of the condition at birth in the whole population. No data are on hand, however, to prove the point.

Since in this study only litters in which all pigs were raised could be used, it is interesting to compare the frequency with which the different litter sizes occur with that shown by the experimental litters. Such a comparison is presented in Figure 1. In making this comparison the Department of Agriculture data have been rated up to the same total as the Chester White. However unsafe such a procedure may be, it gives rise to some interesting speculations as to what the breeder may have done which will affect the results reported in this paper.
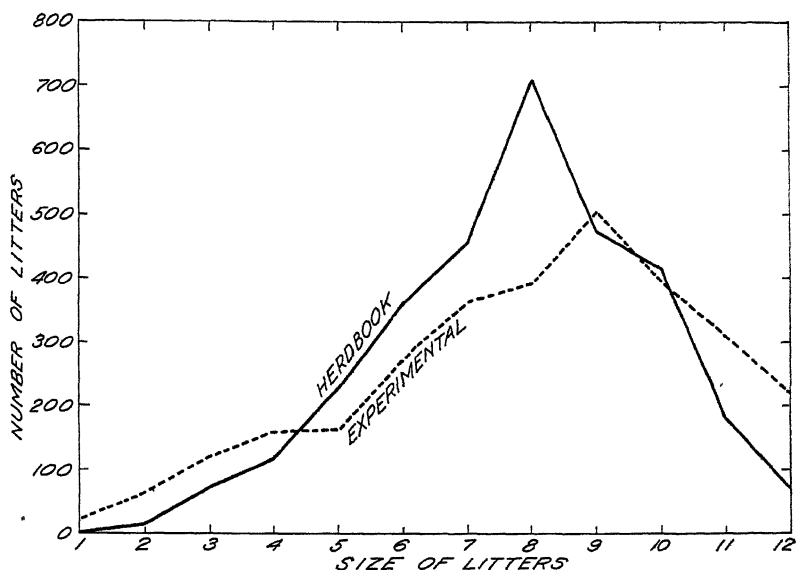
FIG. 1.—Frequency of different litter sizes as shown by herdbook data and by records of experimental herds. The total number of litters is the same in each lot. The herdbook litters are fewer than expected in the extreme sizes and far in excess of expectation in the medium sizes

It is seen that the herdbook data show fewer litters of 1 to 4 and of 9, 11, and 12 pigs, than do the experimental data. From this it may be inferred that fewer animals from small litters were registered than should have been to make the proportion of these litters representative. Among the larger litters it is not surprising, however, that there should be a deficiency, since only litters in which all pigs were raised have been used, and it is a matter of common knowledge that as litters increase in size beyond five or six the difficulties of avoiding mortality in raising the pigs become greater. However, since all the pigs are supposed to have been raised, this factor should not affect the present data. The frequency of litters of 8 in the herdbook data is almost double that in the experimental data. This fact is difficult to explain on any other ground than a misinterpretation of litter size; that is, the litters which are reported as "8, all raised," are probably in some cases the remainders of larger litters or combinations of two or more small ones. Just how such a hit-and-miss procedure could lower the sex ratio is not clear, however,

since Parkes (*5*) has shown that there is only a very low negative correlation between size of litter and proportion of boars.

It should be kept in mind that the herdbook data used included only litters of 2 to 12 and 2 to 14, while the experimental data include litters ranging from 1 to 20. The omission of the larger-sized litters from the herdbook material could not lower the proportion of boars through the relation between sex and size of litter, however, because the correlation is a negative one and the tendency would be to raise the proportion.

In conclusion, it may be said that so far as the sex ratio is concerned the herdbook data give a ratio which is significantly less than that given by a large body of experimental data. The reasons why the sex ratio is lower in the herdbook data are not clear, but indications point most strongly to errors introduced in reporting and recording the sex of the pigs. There is no indication that the condition has any biological explanation.

FREQUENCY OF SEX COMBINATIONS

In swine, where multiple births are the rule, a variety of sex combinations may occur. The number of different possible combinations in any litter size is given by the formula $n^{+}1$, in which n is the size of the litter. If sex determination is a random process, the distribution of such sex combinations in each litter size should be given by the expansion of the binomial $N(p+q)^n$, in which N is the number of litters of any given size, n is the number of pigs in the litter, and p and q are the proportions of males and females, respectively.

In testing the closeness of agreement between the observed frequencies and those given by the binomial expansion, use can be made of Pearson's chi-square test for goodness of fit. The ordinary formula for calculating χ^2 is $\chi^2 = S\left(\dfrac{x^2}{m}\right)$, where m is the expected number and x is the difference between expected and observed. Extensive tables are available which give the values of P for the different values of χ^2 and n. The method gives a value for P which shows the probability in future trials of obtaining a greater value for χ^2 than the one observed. Its use in this paper is to determine whether the differences between the observed and calculated values are caused by sampling or by factors affecting the distribution of sex frequencies. It is obvious that as the value of χ^2 increases for any value of n the value of P decreases. It becomes necessary, therefore, to select some arbitrary range in the scale of values within which it may be said that the value of P indicates an unquestioned agreement between observed and calculated values. According to Fisher (*4*) such a range is from 0.1 to 0.9. That is, if it is assumed as a working hypothesis that the frequencies of sex combinations in the different litter sizes are given by the expansion of the binomial $N(p+q)^n$, a value for P of something less than 0.1 must be obtained before the hypothesis can be called in question and a value of less than 0.05 must be obtained before it is a certainty that the hypothesis does not fit the observed values.

TABLE 3.—*Values of P for testing goodness of fit in Department of Agriculture, Chester White Swine Record, and National Duroc-Jersey Record data*

Size of litter	Depart- ment of Agricul- ture data	Chester White Swine Record	National Duroc- Jersey Record
2	0.1952	0.8900	0.7303
3	.9665	.3926	.7314
4	.6831	.0481	.2538
5	.2623	.3433	.8277
6	6906	.0221	.3366
7	.9160	.7707	.3192
8	.8378	.0002	0001
9	.4995	.0000	.0759
10	.6678	0054	0017
11	8665	.0197	.2112
12	.1652	.0125	.1298

In Table 3 are shown the values of χ^2 and P for testing agreement between the observed and calculated frequencies of sex combinations in litters of 2 to 12, inclusive, of the Department of Agriculture, Chester White Record, and National Duroc-Jersey Record data. The calculated and observed frequencies in the Department of Agriculture and Chester White Record data are also shown in Tables 4 and 5, respectively. If Fisher's (4) range in values for P is taken as indicating agreement between observed and calculated, it may be said that in litters of 4, 8, 9, 10, 11, and 12 of the Chester Whites and litters of 8, 9, and 10 of the Duroc-Jerseys, the observed frequencies show such marked divergence from the calculated as to indicate either a serious fallacy in the hypothesis or faults in the data. In view of the fact that herdbook data give good fits in some litter sizes, it seems probable that the data are at fault. This becomes more apparent when the values of P for the Department of Agriculture data are examined. It is seen that the range in values of P for the experimental data is from 0.165 to 0.966, and it may be said that such values lie within the expected range. It is particularly important to note that in litters of 8, 9, and 10 the values of P are 0.8378, 0.4995, and 0.6678, respectively, thus showing good fits. Since it is in these three litter sizes that the herdbook data show exceedingly wide departures from expectation, this result becomes very important, indicating that there is a very serious defect in this portion of the herdbook data. In view of the fact that it was in these same litter sizes that a considerable discrepancy has been noted between the numbers of experimental and herdbook litters, it seems desirable to analyze the situation further.

TABLE 4.—*The observed and calculated frequencies of sex combinations, together with value for goodness of fit for Department of Agriculture experimental data; calculated on the basis of mean proportion of males*

Number of males	2		3		4		5		6		7		8		9		10		11		12	
	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated
0	1	3.9	3	3.5	2	2.6	1	1.2	1	0.9	0	0.6	0	0.3	0	0.2	0	0.1	0	0	0	0
1	10	8.5	12	11.5	8	11.3	4	6.3	4	5.9	4	4.4	5	2.6	3	1.9	1	.8	0	.3	0	.1
2	6	4.6	12	12.5	15	12.2	12	13.7	13	15.9	12	13.3	9	9.8	5	8.3	6	3.7	1	1.7	0	.7
3			5	4.5	13	13.3	13	14.8	28	23.0	30	25.9	22	21.3	20	21.0	5	10.6	3	5.6	3	2.7
4					5	3.6	12	6.3	17	18.7	25	28.0	25	28.8	36	30.1	21	20.1	14	12.1	8	6.5
5							2	1.7	8	8.1	18	18.2	26	24.9	38	32.6	30	26.1	19	18.3	10	10.2
6									3	1.5	8	6.6	14	13.5	21	26.7	22	23.6	21	19.8	13	12.9
7											1	1.0	4	4.2	10	12.4	14	14.6	17	15.3	11	12.0
8													1	.6	4	3.6	7	5.9	7	8.3	8	8.9
9															0	.4	1	1.4	3	3.0	2	4.3
10																	0	.2	0	.6	3	1.4
11																						.3
Total	17	17.0	32	32.0	43	43.0	44	44.0	74	74.0	98	98.0	106	106.0	137	137.2	107	107.1	85	85.0	60	60.0
X^2	0.1687		3.3178		2.2960		6.5129		3.8946		2.0330		3.4484		6.3632		5.8317		3.1857		11.7226	
X	0.1952		0.9665		0.6831		0.2623		0.6906		0.9160		0.8378		0.4995		0.6678		0.8655		0.1652	

TABLE 5.—*The observed and calculated frequencies of sex combinations, together with value for goodness of fit for Chester White herdbook data; calculated on the basis of mean proportion of males*

Number of males	2		3		4		5		6		7		8		9		10		11		12	
	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated	Ob-served	Calcu-lated
0	3	3.7	6	9.8	8	8.1	8	8.2	1	6.7	1	4.2	2	3.5	2	1.3	0	0.5	0	0.1	0	0
1	8	7.0	29	27.8	44	30.7	29	38.5	30	37.9	26	28.6	22	26.5	3	10.8	4	5.1	9	1.2	0	.3
2	3	3.3	31	26.2	37	43.4	76	72.6	99	89.3	87	80.8	71	87.4	23	40.7	19	21.4	9	5.9	0	1.5
3			6	8.2	19	27.3	65	68.4	120	112.2	126	126.9	165	164.7	86	89.5	38	53.9	10	16.5	8	4.7
4					8	6.4	42	32.2	74	79.3	118	119.8	245	194.0	177	111.5	111	88.8	37	31.2	7	10.0
5							6	6.1	26	29.2	73	67.7	151	146.3	100	105.0	102	100.5	42	41.2	18	13.8
6									10	4.7	19	21.3	44	68.9	51	75.0	87	78.9	45	38.8	16	15.1
7											2	2.8	10	18.5	25	30.3	40	42.5	20	26.1	7	12.2
8													2	2.2	2	7.1	4	15.0	10	12.3	7	7.9
9															3	.7	5	3.1	4	3.9	3	3.3
10																	0		0	.7	4	1.0
11																			1	.1	0	.2
12																					0	0
Total	14	14	72	72	116	115.9	226	226	360	359.3	452	452.1	712	712.0	472	472	410	410	178	178	70	70
X²	0.3026		2.9950		9.6303		5.6675		14.061		4.0754		30.9654		73.5811		21.7789		18.2322		17.8951	
P	0.8900		0.3926		0.0481		0.3433		0.0221		0.7707		0.0002		0.0000		0.0054		0.0197		0.0125	

The standard deviations which are shown in Table 6 indicate that grouping into certain sex combinations is an important factor in bringing about the condition which has resulted in such marked disagreement between observed and calculated values. In the Chester White data the observed standard deviations are less than the calculated in all litter sizes studied except 4, 7, and 12. Parkes (5) found that in the Duroc-Jersey herdbook data the observed standard deviations were less than the calculated except in litters of 11 and 12. His figures are included in Table 6 for the purpose of comparison. Now it is important to note that although these differences exist, not all of them are of such magnitude as to be significant statistically. Among the Chester Whites only in litters of 8, 9, and 10 are the differences between the observed and the calculated standard deviations as much as or more than three times the probable error, and therefore only in these three litter sizes can it be stated definitely that we are dealing with differences, which are not caused by the fluctuations of random sampling. Likewise among the Duroc-Jerseys it is in litters of 8, 9, and 10 that the greatest differences are observed, and although Parkes (5) does not give the probable errors, an examination of the magnitude of the differences will show that only in these three litter sizes are the differences significant. Then it may be said that the two groups of data are essentially similar in this one respect. Among the experimental litters, on the other hand, the situation seems to be different in that there are no significant differences between the observed and the calculated standard deviations. Furthermore, there seems to be no regularity with respect to the cases in which the observed is less than the calculated.

TABLE 6.—*Observed and calculated standard deviations for frequency of sex combinations in each litter size of Department of Agriculture, Chester White Swine Record, and National Duroc-Jersey Record data*

Size of litter	Department of Agriculture data		Chester White Swine Record		National Duroc-Jersey Record	
	Observed σ	$\sqrt{npq}$	Observed σ	$\sqrt{npq}$	Observed σ	$\sqrt{npq}$
2	0. 5703	0. 6758	0. 6547	0. 7071		
3	.8609	.8643	.7636	.8659		
4	1. 0421	.9918	1. 0239	.9941	0. 824	0. 999
5	1. 1272	1. 1114	1. 1173	1. 1178	1. 069	1. 118
6	1. 2171	1. 2207	1. 1737	1. 2247	1. 103	1. 224
7	1. 2952	1. 3204	2. 7574	1. 3224	1. 253	1. 303
8	1. 4886	1. 4133	1 2502	1. 4132	1. 194	1. 414
9	1. 4492	1. 4993	1. 3257	1. 4636	1. 350	1. 499
10	1. 5562	1. 5799	1 4415	1. 5804	1. 430	1. 581
11	1. 4966	1. 6564	1. 6037	1. 6541	1. 688	1. 658
12	1 9018	1. 7320	1. 8615	1. 7315	1. 769	1. 731

What do these conditions mean with respect to the departure from expectation in distribution of the different sex combinations? First of all, a grouping into the mean sex combinations is clearly evident in both groups of herdbook data and just as clearly absent from the experimental data. From the experimental data we observe that the frequencies of the different sex combinations are essentially those given by the expansion of the binomial $N(p+q)^n$. In litters of 8, 9,

and 10 of herdbook data this relation does not hold, however, and reference to Table 5 will reveal the reason why. There is a noticeable surplus of litters in the mean sex combinations and a deficiency of litters in the extreme sex combinations. Such a condition can come about only through a failure to report the actual condition at birth. The tendency has been to report a large proportion of the litters as containing an equal number of males and females, which fact strongly suggests a too extensive use of the human memory as a record book. The causes which limit the deficiencies to litters of 8, 9, and 10 are probably economic ones. First of all, these particular litter sizes are among those which occur most frequently, and hence it may be expected that they would offer the greatest opportunity for the introduction of irregularities. Secondly, the sizes are such as to be easily synthesized by combining two smaller litters and registering as one. This would account for at least a part of the excess of litters of 7 and 8, and the deficiency of litters of 2, 3, and 4 in the herdbook data. Of course certain litters born as 12 or more and in which only 8 or 9 were raised might be registered as the number raised. There are other possibilities as well, but little is to be gained from discussing them. One thing seems to stand out very clearly, that the process of introducing the defects is essentially as complicated as human nature, and hence the possibility of rectifying the errors by a process of correction is not only remote but practically impossible. This is exceedingly regrettable because the only value which swine herdbook data have or probably ever will have for research purposes consists of that which pertains to the sex of the pigs and the size of the litter. There can be little doubt that in both these respects the data are too unreliable to be of any real value.

In this connection it may be well to lay particular emphasis on the point that the results reported in this paper concerning the value of herdbook data for investigational purposes apply to swine herdbook data in general and not to those of any one breed. The defects appear to be of the same general nature in both the Chester White and the Duroc-Jersey breeds. In other words, the fact that the Chester White Swine Record has been used in the present study and has been found to be defective in certain respects should not be interpreted as meaning that the Chester White Swine Record is any more inaccurate or incomplete than are the records of the other breeds.

SUMMARY AND CONCLUSIONS

The proportion of males in swine at birth as shown by three lots of independent experimental data is approximately 0.52.

The proportion of males found in herdbook data is too low by between 3 and 4 per cent.

In the experimental data the frequency with which the different sex combinations in each litter occurred are in agreement with those given by the expansion of the binomial $N(p+q)^n$, in which N is the number of litters and n the size of the litter.

Herdbook data do not possess the necessary accuracy for a study of sex ratio and frequency of sex combinations. The most apparent defects seem to be a failure to report and record accurately the sex of pigs and the size of litter at birth. These defects are not peculiar to the records of any one breed association.

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RELATION OF SIZE OF OIL DROPS TO TOXICITY OF PETROLEUM-OIL EMULSIONS TO APHIDS[1]

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INTRODUCTION

Petroleum oils, largely in the form of miscible oils or of emulsions, are used to control scales and other insects on fruit trees. A miscible oil contains a dissolved emulsifier, which causes emulsification when the oil is properly diluted with water. Sulphonated oils, the material obtained in the refining of petroleum, and similar emulsifiers are soluble in oil. Others, such as soap, are made to dissolve in oil by the addition of a third substance like cresylic acid. Usually the miscible oils contain a little water and are clear and oily in appearance.

Some miscible oils mix readily with water when poured directly into it. Others must have a little water stirred into them before they can be further diluted. If water is poured directly into a miscible oil of the second type an inverted or water-in-oil emulsion, which floats on the surface of the water, is formed. When properly diluted, however, miscible oils of either type form milky emulsions in which the oil drops are very small, usually less than 2 microns in diameter. Such diluted emulsions cream out slowly on standing, and are very resistant to breaking by hard water.

The oil drops in oil emulsions are usually larger than those in diluted miscible oils. The size depends on the emulsifier used and on the method of preparation. Oil emulsions, when diluted with water, cream much more rapidly than the emulsions resulting from the dilution of miscible oils. Those made with a soap emulsifier are much less resistant to breaking in hard water than are diluted miscible oils.

It has been the usual experience that a miscible oil diluted for spraying requires a larger percentage of oil than a lubricating-oil emulsion. Miscible oils for dormant spraying are generally used at a dilution of about 1 gallon to 15 gallons of water and contain from 80 to 90 per cent of petroleum oil. At the 1 to 15 dilution they would have an oil concentration of approximately 5 per cent. Lubricating-oil emulsions for dormant work have usually had a concentration of 2 or 3 per cent oil. This discrepancy in dosage has probably sometimes resulted from the use of oils lighter and less toxic than the lubricating type. No reports of experiments with miscible oils and emulsions made from the same petroleum oil have been found in the literature. Jarvis (*6*)[2] and Houghton (*5*), who used a paraffin oil of lubricating type in some of their preparations, showed that from 5 to 10 per cent of oil was necessary for satisfactory control of the San Jose scale.

The main differences between a diluted miscible oil made with cresylic acid and soap and a diluted soap emulsion appear to be the presence in the former of cresylic acid and the difference in the size of the oil drops. It is conceivable that either of these factors might cause a difference in toxicity.

The cresylic acid might tend to lower the toxicity by either of two methods. First, it has been shown (*3*) that a petroleum oil-soap emulsion consists of droplets of oil, each surrounded by a film of soap and suspended in an aqueous medium. The cresols have a tendency to form molecular compounds or addition products with many organic compounds. They might form such compounds with the soap in the films surrounding the oil droplets, resulting in tougher films, which would prevent the oil from readily coming in contact with the surface of the plant being sprayed or with the insect to be killed. Secondly, it is conceivable that cresylic acid, in small proportions, might act physiologically on the insect, making it less susceptible to the toxic action of the oil.

Recently de Ong (*1*) has carried out studies which indicate that the permanent or stable type of emulsion is not so efficient for insecticidal purposes as a quick-breaking form—that is, one in which the oil separates out very quickly from the dispersed phase in the water. He suggests that the ideal form of insecticidal emulsion is one which approaches the mechanical type of emulsion, in that the droplets of oil are comparatively large and separate from the water almost immediately on striking the leaf surface.

The permanence or stability of an emulsion is very closely linked with its drop size, the emulsion with small oil drops being most permanent. This suggests that the lower toxicity of the miscible oils might be due to the small drop size as compared with oil emulsions.

When a spray is applied to a plant some of the liquid runs or drains off. The oil in the portion which drains off is lost. Only the portion which remains fixed is of value as an insecticide. The spray which drains off may contain a higher or lower percentage of oil than that first applied, depending on whether the oil or the water is more readily absorbed by the plant surface.

Moore (*7*) states that the electrical charge exhibited by a leaf surface (and presumably by most other external plant structures) should be negative because filter paper, consisting of cellulose, is negative, and cotton, which is chiefly cellulose, is also negative. He confirms this conclusion by showing that stains which give good results with leaf tissues are positive. As they are readily absorbed by the leaf tissue, that tissue must be negatively charged. To definitely settle the question he carried out endosmosis experiments with bean and citrus leaves. When a leaf diaphragm was placed between the two electrodes and the current was turned on, water migrated through the leaf in the direction of the negative electrode, showing that the leaf surfaces when wet bore a negative electrical charge.

The charge (*2*) on the suspended droplets of oil in an oil emulsion is usually negative. This negative charge would tend to prevent the oil from adhering to the negatively charged leaf or other similarly charged plant surface. The charge is naturally a surface phenomenon, and, other things being equal, it will be proportional to the area present. The area per unit volume of any droplet is its area

divided by its volume, or, as the oil droplets are spheres or nearly spheres, $\frac{4\pi r^2}{4/3\ \pi r^3} = \frac{3}{r}$, r being the radius. Thus the surface, and also the electrical charge associated with any volume of oil, are inversely proportional to the radius of the droplets into which the oil is divided. When the oil is dispersed into droplets with a radius of 0.5 micron, 10 times the charge is associated with the same volume of oil as when it is dispersed into droplets with a radius of 5 microns. This leads to the conclusion that the forces which tend to keep the oil from adhering to plant surfaces must be much greater in an oil dispersed in small droplets than in one dispersed in large droplets. Consequently, the proportion of oil held by a plant surface should be lower when the droplets are small, making the emulsion less toxic to insects than an emulsion with large oil droplets.

Experiments were undertaken to determine whether the toxicity of petroleum oils to insects could be correlated with the size of the oil droplets and, if so, to ascertain the reason.

EXPERIMENTAL PROCEDURE

MATERIALS

Ten petroleum oils, ranging from a light, volatile oil of low viscosity to a lubricating oil of high viscosity, and representing both the paraffin and naphthene base oils, were used. Their properties are given in Table 1.

TABLE 1.—*Properties of the experimental oils*

Sample No.	Oil	Base	Color	Density, 20° C.	Flash point	Fire point	Volatility (4 hours at 105° C.)	Viscosity (Saybolt, 100° F.)	Unsulphonated
				Grams per c.c.	° F.	° F.	Per cent	Seconds	Per cent
1	Kerosene		Light straw	0.811	130	150	58.2	31	83
2	Gas oil		Black	.879	170	205	23.3	50	64
3	Red engine oil (IV)	Paraffin or mixed.	Red	.902	400	455	.2	231	61
4	Liquid petrolatum	Paraffin (?)	White	.879	390	425	.2	218	97
5	Paraffin oil 115	Paraffin	Yellowish brown.	.883	360	400	.4	110	67
6	Paraffin oil 222	do	Reddish brown	.896	380	425	.3	218	68
7	Paraffin oil 318	do	Yellowish brown.	.897	415	475	.06	313	76
8	Naphthene oil 100	Naphthene	do	.912	305	335	1.6	105	63
9	Naphthene oil 202	do	Reddish brown	.919	335	365	.7	200	60
10	Naphthene oil 305	do	Brown	.924	345	380	.6	298	60

Sample 3 was a commercial red engine oil, probably a mixed base oil, that has been widely used to control insects on citrus and deciduous fruit trees. Sample 4 was a well-known brand of white medicinal oil. The viscosities of samples 5, 6, 7, 8, 9, and 10 (lubricating oils) were approximately 100, 200, and 300 seconds for each base (paraffin and naphthene).

The soap used was a commercial fish-oil soap of potassium base, having the following composition: Water, 62.8 per cent; fatty anhydrides, 28.5 per cent; potassium oxide, 5.8 per cent; glycerol, etc. (by difference), 2.9 per cent. The cresol was of United States Pharmacopoeia grade.

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The size of the oil droplets was determined under the microscope by means of an eyepiece micrometer. A drop of dilute sodium hydroxide solution was added to prevent the agglutination of the droplets. These measurements are intended to give only the approximate average or usual size of the droplets, but are considered sufficiently accurate for the purposes of this investigation.

The emulsions were prepared by the following methods:

(1) A soap-oil emulsion was made either by the Government formula (*9*) or by the cold-mixed method (*8*). In the Government process a mixture in the proportions of 2 gallons of oil and 1 gallon of water, containing 1 quart of potassium fish-oil soap, was heated to incipient boiling, and then forced three times through a disk-type spray nozzle at a pressure of 40 pounds per square inch. In the cold-mixing process the oil was stirred into the soap in the same proportions, and water was then added. The usual drop size in either case was about 8 to 10 microns. A part of the emulsion obtained by one of these methods was passed several times through a colloid mill, the usual diameter of the drops in the resulting emulsion being about 2 microns.

(2) An emulsion was prepared by stirring cresol into potassium fish-oil soap, and stirring the oil into the resulting mixture (*8*). The proportions used were 2 gallons of oil, 1 quart of fish-oil soap, 8 fluid ounces of cresol, and 5 pints of water. The usual diameter of the drops in this emulsion was 2 microns or less. An emulsion containing the same ingredients in like proportion, but having drops of a diameter of 8 to 10 microns, was obtained by diluting the cold-stirred emulsion of soap and oil with water containing 0.5 per cent of cresol.

(3) An oil-water emulsion was prepared by passing a mixture of approximately 1 part of oil and 10 parts of water several times through a colloid mill. After the grinding the oil was largely in droplets about 10 microns in diameter. The emulsion so obtained was tested against the insects. A portion of this emulsion was allowed to stand for 24 hours. It had then separated into two layers, the droplets in the lower layer being mainly 2 microns or less in diameter.

Thus three pairs of preparations, the members of each pair having the same chemical composition, but differing in drop size, were obtained.

The foregoing proportions were taken in making all the preparations, but slight variations due to the adherence of the oil to the walls of the glassware used in measuring, to temperature changes, to foaming, and to other causes occurred. The approximate oil concentration (per cent by volume) was determined in each emulsion after preparation by a modified Babcock method (*4*). The dilutions used in the tests on aphids were made on the basis of these determinations.

SPRAYING METHODS

Aphis rumicis L., reared on dwarf nasturtium plants (*Tropaeolum majus*) in a greenhouse, was employed for these experiments. For each experiment portions of plants holding groups of aphids of all ages were used, the stems being immersed in water in a bottle. The plants were thoroughly sprayed, the surplus drops of liquid being removed by gentle shaking. The bottle with its contents was then

placed upon a sheet of white paper, the edges of which were coated with adhesive material to prevent the escape of living aphids. The spraying was done with an atomizer, under an air pressure of 5 pounds per square inch delivered from a compressed air line through an adjustable reducing valve. The percentage of dead aphids was determined at the end of 18 to 26 hour periods. In most instances, however, the time was from 20 to 24 hours, which is much longer than the period necessary to produce the maximum number of deaths at the concentration used. The temperature and humidity were not controlled.

At the conclusion of each experiment the aphids were counted and the percentage of dead individuals was determined. Three experiments were made for each concentration of oil. From 802 to 1,939 aphids were used for each set of three experiments, the number falling below 1,000 in only 4 of the 38 sets of experiments. The weighted mean percentage of dead aphids was determined for each set of experiments, and from this the mean deviation (per cent) was calculated. This simple method of evaluating deviation was considered sufficiently exact for the purposes of this investigation.

EXPERIMENTAL RESULTS

TOXIC EFFECT OF DILUTED EMULSIONS

The action of the petroleum-oil emulsions upon *Aphis rumicis* is shown in Table 2, and in Figures 1 and 2.

TABLE 2.—*Toxic action of oil emulsions on Aphis rumicis*

Preparation No.	Oil	Emulsifier	Method of emulsification	Size of oil drops (Microns)	Concentration of oil by volume (Per cent)	Total number of aphids (3 experiments)	Aphids killed (Per cent)	Mean deviation of percentage killed (Per cent)
112	Kerosene	Potassium fish-oil soap	Hot, 40-pound pressure	8	1.0	1,050	96.7	±3.60
				8	2.5	962	99.2	±.35
77,146	do	Potassium fish-oil soap and cresol	Cold-stir method	4	1.0	1,533	41.9	±15.98
117	do	Potassium fish-oil soap	Mixed by cold-stir method; ground in colloid mill	4	2.5	1,168	89.7	±6.57
116	Gas oil	do	Hot, 40-pound pressure	7-8	.5	1,376	20.3	±9.64
			do	7-8	1.0	1,036	85.3	±4.78
119	do	Potassium fish-oil soap and cresol	Cold-stir method	2	1.0	1,175	98.7	±6.73
148	Red engine oil IV	Potassium fish-oil soap	do	2	2.5	1,219	54.7	±6.71
151	do	do	Cold-stir method; cresol added on dilution	10	.5	1,430	92.5	±.91
152	do	do	Prepared from 148 by grinding in colloid mill	10	.5	1,248	92.3	±3.41
154,155	do	None	Oil and water ground in colloid mill	8	.5	1,266	90.3	±7.92
154-a	do	do	do	8	.5	1,735	46.5	±4.49
113	Liquid petrolatum	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	a 1,939	89.5	±1.02
74,145	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,505	70.6	±8.44
			do	2	.5	1,053	97.3	±1.68
				2	1.0	1,149	61.5	±12.94
133	Paraffin oil 115	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	1.0	1,128	93.6	±1.79
139	do	Potassium fish-oil soap and cresol	Cold-stir method	2	1.5	1,189	96.0	±2.47
134	Paraffin oil 222	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,348	97.0	±2.14
140	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,225	77.8	±1.87
155	do	Potassium fish-oil soap	Cold-stir method; ground in colloid mill	2-3	.5	1,250	99.2	±.95
157	do	do	Cold-stir method; cresol added on dilution	8-10	.5	1,259	57.2	±13.90
158	do	None	Oil and water ground in colloid mill	2	.5	1,164	71.7	±7.36
158-a	do	do	do	10	.5	1,297	94.7	±3.21
135	Paraffin oil 318	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,170	94.6	±3.69
141	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	935	74.9	±3.32
188	Naphthene oil 100	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,330	98.0	±1.30
144	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,371	55.7	±7.52
137	Naphthene oil 202	Potassium fish-oil soap	Hot-40-pound pressure	8-10	.5	1,257	98.6	±1.25
143	do	do	Cold-stir method; ground in colloid mill	2	.5	1,468	65.6	±6.13
159	do	Potassium fish-oil soap and cresol	Cold-stir method	8-10	.5	1,174	97.5	±2.07
161	do	Potassium fish-oil soap	Cold-stir method; ground in colloid mill	2	.5	1,367	60.3	±8.54
160	do	do	Cold-stir method; cresol added on dilution	10-12	.5	892	80.7	±11.30
160-a	do	None	Oil and water ground in colloid mill	10	.5	802	80.1	±1.25
	do	do	do	2	.5	1,063	95.9	±3.47
135	Naphthene oil 305	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	.5	1,334	98.7	±3.10
142	do	Potassium fish-oil soap and cresol	Cold-stir method	2	.5	1,064	55.9	±.59
				2	.5	1,373	61.0	±5.25

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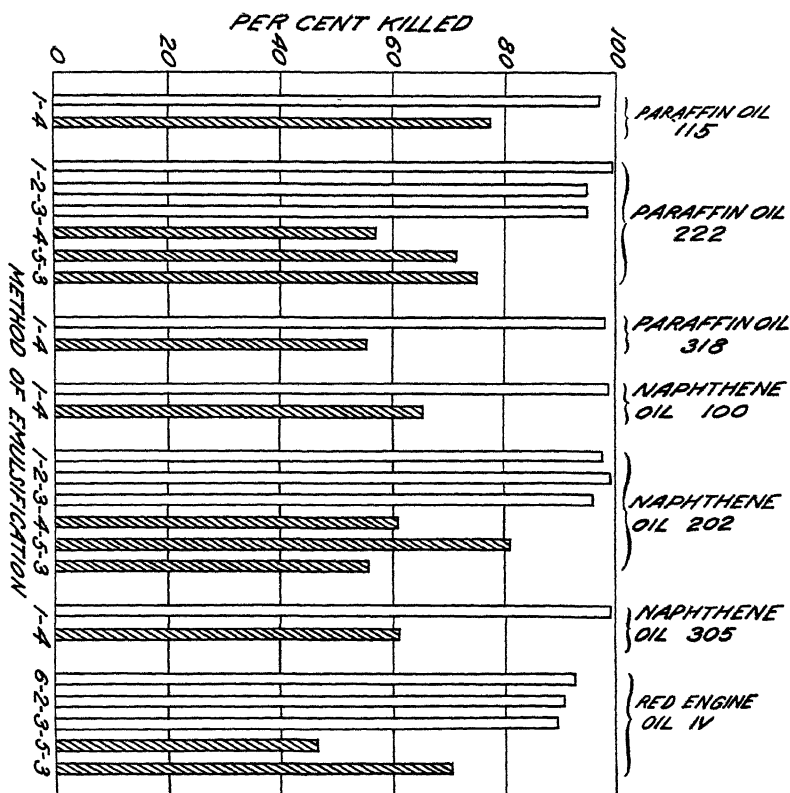
Fig. 1.—Toxicity to *Aphis rumicis* of lubricating-oil emulsions containing 0 5 per cent oil, showing the comparative effect of large and small drop size. Crosshatching indicates small oil drops

Method of emulsification· (1) Boiled soap oil-emulsion; (2) cold-mixed emulsion without cresol, cresol added on dilution; (3) oil and water ground in colloid mill, no soap; (4) cold-mixed emulsion with cresol, (5) cold-mixed emulsion without cresol, ground in colloid mill; (6) cold-mixed emulsion without cresol.
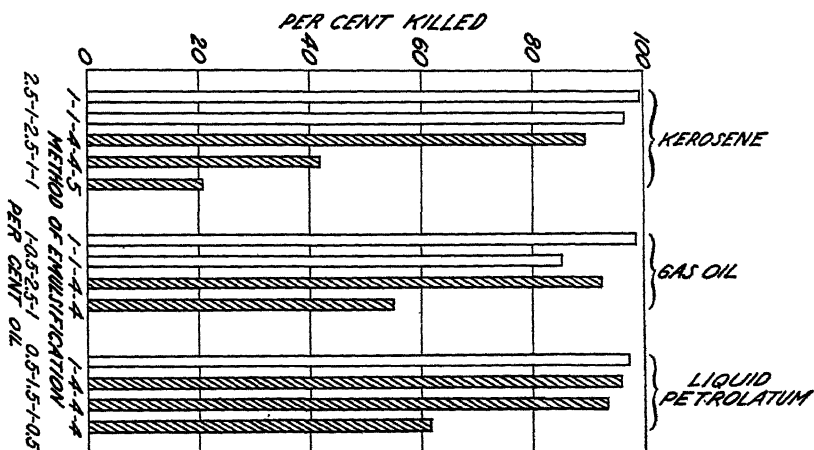
Fig. 2.—Toxicity of light and heavy oils to *Aphis rumicis*, showing effect of concentration and drop size. Crosshatching indicates small oil drops

Method of emulsification: (1) Boiled soap-emulsion; (4) cold-mixed emulsion with cresol; (5) cold-mixed emulsion without cresol, ground in colloid mill.

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When the diameters of the drops lay between 8 and 12 microns, the oils of the lubricating type killed 90 to 100 per cent of the aphids at an oil concentration of 0.5 per cent. This was true for oils of paraffin and naphthene base, for oils ranging in viscosity (Saybolt) from 105 to 313 seconds, and for oils that contained a variable quantity of constituents reactive with sulphuric acid. (Table 1.) The method of preparation of the emulsion had little influence on toxicity (red engine oil IV, paraffin oil 222, and naphthene oil 202). The results obtained with red engine oil IV in an emulsion in which the droplets were large are slightly lower than the toxicity figures for the other high-viscosity oils. This result is probably not characteristic for emulsions of this oil in which the drop size is large, for some other preparations made with this oil, and not included in this investigation, have given higher toxicity at the same oil concentration.

When the drop size is small (2 or 3 microns or less in diameter), the toxicity of the emulsions containing lubricating oils is significantly lowered, the ratio of aphids killed falling within the range of 40 to 80 per cent. Table 1 and Figure 1 show that this low toxicity persists, regardless of the base, viscosity, or other physical characteristics of the oil, and that it is not altered by the method of preparation of the emulsion. That the presence of cresol does not influence toxicity, either directly by its action upon the aphids or indirectly through some effect upon the emulsion, is also brought out by these experiments. The emulsions of oil and water mixed by grinding in the colloid mill gave toxicities that fall within the range of the toxicities produced by emulsions containing cresol, the drop size in both preparations being about 2 microns. Furthermore, when cresol is added to emulsions of large drop size (8 to 12 microns), their toxicities are not appreciably changed.

The difference in toxicity between the emulsions of kerosene and of gas oil containing relatively large oil droplets (7 to 8 microns in diameter) and those containing relatively small droplets (2 to 4 microns in diameter) is clearly indicated in Table 2 and Figure 2. In experiments with 1 and 2.5 per cent kerosene, the emulsion having been prepared by the cold-stir method with cresol, the drop size was larger than usual (4 microns). This may account for the relatively high toxicity (89.7 per cent) obtained with a concentration of 2.5 per cent of this oil as compared with the toxicity from an emulsion applied at the same oil concentration, but in which the oil droplets were 8 microns in diameter. A similar relation is shown by a comparison of a kerosene emulsion, made by the cold-stir method without cresol and ground in the colloid mill to a drop size of 2 microns, with an emulsion, similarly prepared but containing cresol, in which the drop size was 4 microns. At the same oil strength (1 per cent by volume), the second emulsion was twice as toxic as the first.

The results from the experiments with gas oil show even more distinctly the effect of drop size on toxicity. The toxicity of the emulsion with large oil droplets (7 to 8 microns) is approximately two times greater than that of the emulsion containing droplets of small size (2 microns), when the same concentrations are compared.

The emulsions containing the more volatile oils (kerosene and gas oil) were, as has generally been observed, less toxic than the mixtures

made with oils of the lubricating type, requiring two or more times the concentration of oil to produce the same effect.

The experiments with emulsions containing liquid petrolatum indicate that when the drop size is 2 microns or less, 95 per cent or more of the aphids are killed only when the oil concentration is raised to about three times that required of emulsions containing large oil droplets (8 to 10 microns). The experiments with kerosene and gas-oil emulsions show a similar relation.

RELATION OF DROP SIZE TO RETENTION OF OIL BY SPRAYED PLANTS

Emulsions in which the oil droplets were large spread readily over the foliage and stems, and, upon evaporation of the aqueous portion, left a nearly continuous coating of oil on them. This film of oil was indicated by the dark color of the sprayed foliage. Emulsions in which the oil droplets were relatively small spread poorly. The liquid collected in drops on the sprayed foliage and stems, and, upon evaporation of the water, left no continuous coating of oil, but only scattered oily spots.

Efforts were made to show this relation quantitatively. Plants were sprayed with diluted emulsions of known oil concentration. The emulsion that dripped from the plant during the spraying operation was collected, and the quantity of oil remaining on the plant was determined. The oil concentrations in the original dilute emulsion and that collected from the plants were determined by the modified Babcock method (4). Approximately the same quantity of nasturtium and bean foliage and of apple and peach twigs (without leaves) was used in each test. Nearly the same quantity of dilute emulsion was applied at a pressure of 5 pounds. The results shown in Table 3 are estimates only of the quantity of oil retained by the plants under these conditions.

TABLE 3.—*Quantity of oil retained by sprayed plants*

Preparation No.	Plants	Oil	Emulsifier	Method of emulsification	Size of oil drops	Concentration of oil by volume		Oil remaining on plants	Mean values (based on last column)
						Before application	In drip from plants		
					Microns	*Per cent*	*Per cent*	*Per cent*	*Per cent*
118	Nasturtium plants	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap	Hot, 40-pound pressure	10	0.95	0.6	36.8	--------
137	do	Naphthene oil 202	do	do	8-10	2.02	1.73	14.3	--------
137	do	do	do	do	8-10	2.02	1.73	14.3	21.8
121	do	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap and cresol.	Cold-stir method	2	1 00	.90	10.0	--------
143	do	Naphthene oil 202	do	do	2	1.92	1.92	0	--------
143	do	do	do	do	2	1.92	1.92	0	3.3
118	Bean plants	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap	Hot, 40-pound pressure	10	.95	.60	36.8	--------
118	do	do	do	do	10	2 00	1.40	30.0	--------
137	do	Naphthene oil 202	do	do	8-10	2.02	1.15	43.1	--------
137	do	do	do	do	8-10	2.02	1.15	43.1	--------
121	do	do	Potassium fish-oil soap and cresol.	Cold-stir method	8-10	2.02	.96	52.5	--------
121	do	Paraffin oil (91 seconds viscosity).	do	do	2	1.00	.90	10.0	41.1
121	do	do	do	do	2	2.00	1.80	10.0	--------
143	do	Naphthene oil 202	do	do	2	1.92	2.02	0	--------
143	do	do	do	do	2	1.92	2.02	0	5.0
137	Peach twigs	do	Potassium fish-oil soap	Hot, 40-pound pressure	8-10	2.02	1.54	23.8	--------
137	do	do	do	do	8-10	2.02	1.63	19.3	21.3
143	do	do	Potassium fish-oil soap and cresol.	Cold-stir method	2	1.87	1.92	0	--------
118	Apple twigs	Paraffin oil (91 seconds viscosity).	Potassium fish-oil soap	Hot, 40-pound pressure	10	1.73	.91	47.4	0
118	do	do	do	do	10	1.73	.91	47.4	--------
121	do	do	Potassium fish-oil soap and cresol.	Cold-stir method	10	1.73	.67	61.3	54.3
121	do	do	do	do	2	1.92	1.92	0	--------
121	do	do	do	do	2	1.92	1.68	12 5	6.2

In every case, more oil remained on the plant after spraying with emulsions having large oil droplets than after spraying with emulsions in which the droplets were small. The higher absorption results obtained with bean foliage and apple twigs appear to be due to the ability of the emulsions to wet these plant surfaces more completely than those of the nasturtium foliage and peach twigs. This difference is possibly due to the different types of plant surface involved, but more extensive and careful studies would be necessary to substantiate such a claim.

SUMMARY AND CONCLUSIONS

Petroleum-oil emulsions were prepared (1) while hot, under pressure, (2) by a cold-stir method, and (3) by grinding in a colloid mill, to give a series of preparations, with or without emulsifiers, in some of which the oil droplets were large (7 to 12 microns in diameter) and in others small (2 to 4 microns or less in diameter).

The emulsions in which the oil droplets were relatively large were decidedly more toxic to *Aphis rumicis* than those in which the droplets were small.

The toxicity of the preparations, as correlated with drop size, was not influenced by the physical characteristics of the oil, the presence or absence of a soap emulsifier, or the presence or absence of cresol.

When foliage or twigs of plants without foliage are sprayed with emulsions of large drop size more oil is retained by the plant surface than when the foliage or twigs are sprayed with emulsions of small drop size. The following explanation for this is offered:

Oil droplets in emulsions and plant surfaces bear negative electrical charges. The droplets in an emulsion of small drop size have a greater charge per unit volume of oil than those in an emulsion of large drop size because the charge is proportional to the surface. It is believed that plant surfaces repel the droplets in the first type of emulsion with a greater force than they repel the droplets in the emulsions of large drop size, and that consequently the electric charges of plant surfaces and oil droplets are a factor in determining the ability of the oil in an emulsion to adhere to plant surfaces.

Under conditions of comparable concentration and type of oil, miscible oils are probably less toxic to insects than the ordinary soap-oil emulsions, because they contain smaller oil droplets and the oil therefore adheres to the plant (and no doubt to the insect) less effectively.

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THREE NEW HYMENOPTEROUS PARASITES OF THE PINE TIP MOTH, RHYACIONIA FRUSTRANA (COMSTOCK)[1]

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The following new Ichneumonidae and Braconidae were reared by the writer and others during a preliminary study of the parasites of the pine tip moth in connection with a project to introduce the parasites of this species into the Nebraska National Forest for the control of the tip moth there.

Campoplex frustranae, new species.

Campoplex frustranae may possibly be one of the many species recently described by Viereck;[2] but in his key to species it runs nowhere convincingly. It corresponds most closely to *angularis* Viereck, but differs in its pale scape and pedicel and in other characters.

Female.—Length 5 mm., antennae 3 mm., ovipositor 1.5 mm. Temples convexly sloping; diameter of lateral ocellus subequal to ocellocular line and slightly more than half postocellar line; eyes shallowly concave opposite antennae, frons only slightly broader than face; combined face and clypeus almost exactly as broad as long; clypeus broadly rounded at apex, medially very slightly produced; malar space about two-thirds basal width of mandible; antennae stout, flagellum thicker in middle than at either end, first joint four times as long as thick, others gradually shorter, those beyond apical fourth as thick as long and submoniliform. Thorax of normal proportions, propodeum with strong carinae, finely granularly opaque except apical areas, which are coarsely transversely rugose, areolar and petiolar area confluent, latter barely concave; areolet small, petiolate, radius beyond middle; nervellus inclivous, broken, but no trace of subdiscoidella present; legs moderately stout; hind basitarsus slightly longer than next two combined. Abdomen rather slender, weakly compressed; first tergite slightly longer than second, this half as long again as third and distinctly longer than broad at apex, spiracle slightly beyond middle; ovipositor sheath more than half as long as abdomen.

Black; scape and pedicel below, mandibles, palpi, front and middle trochanters, and apices of their coxae stramineous; tegulae yellow; front and middle legs otherwise testaceous, their tarsi paler; hind coxae black, reddish at apex; femur ferruginous, tibia brownish stramineous with apex blackish, tarsus fuscous, paler at base; wings hyaline; abdomen immaculate black except slightly reddish apical corners of second tergite.

Male.—Except sexually essentially like female; second tergite more extensively reddish apically.

Type locality.—Falls Church, Virginia.
Host.—*Rhyacionia frustrana* (Comstock).
Type.—Cat. No. 40102, U.S.N.M.

Ten females and 10 males selected from a large series reared by the writer during July, 1924, and April, 1925.

The delicate white cocoon is spun inside the shattered pupal shell of the host.

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[1] Received for publication Jan. 5, 1927; issued June, 1927.
[2] VIERECK, H. L. A PRELIMINARY REVISION OF THE CAMPOPLEGINAE IN THE CANADIAN NATIONAL COLLECTION, OTTAWA. Canad. Ent. 57: 225, 1925; 58: 130, 143–149, 1926.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 8
Apr. 15, 1927
Key No. K–188

Phanerotoma rhyacioniae, new species.

This species is remarkable chiefly for its very strongly depressed abdomen.

Female.—Length 2.7 mm. Head slightly broader than thorax, granularly opaque, sides of frons and upper part of face more or less rugose; eye large, bulging, broadly oval, sparsely hairy, its longest diameter nearly twice the length of ocellocular line; malar space no greater than basal width of mandible; clypeus minutely bidentate at apex; antennae opposite upper fourth of eyes, scape hardly twice as long as thick, basal joint of flagellum about two and a half times as long as thick, joints toward apex changing gradually from cylindrical to nearly round moniliform. Thorax depressed, granularly opaque, notauli irregularly rugose, propodeum medially and apically coarsely irregularly reticulate rugose, laterally more finely so; second abscissa of radius hardly as long as first and distinctly shorter than second intercubitus; first and second abscissae of cubitus not quite continuous, the second cubital cell not pointed at base; nervulus postfurcal by barely its length. Abdomen very flat, the edges especially at apex only slightly rolled downward, apically narrowly truncate but not at all emarginate, finely granularly opaque, longitudinally rather weakly and sparsely striate except in basal middle of first tergite and on apical half of third; first and third tergites subequal in length, second shorter by about a fourth; ovipositor straight, slightly exserted.

Ferruginous; stemmaticum, apices of antennae, propodeum, and third tergite brownish; abdomen otherwise yellowish; coxae, trochanters, and front and middle femora stramineous, hind femur darker; hind tibia reddish below, with apical and subbasal blackish spots above, extreme base and space between spots whitish, the same pattern but less distinct on middle tibia, front tibia mostly dark, tarsi paler than tibiae.

Male.—Like female but eyes larger with malar space and ocellocular space somewhat shorter; subbasal joints of antennae about a half longer than thick.

Type locality.—Bogalusa, Louisiana.
Host.—*Rhyacionia frustrana* (Comstock).
Type.—Cat. No. 40103, U.S.N.M.

Described from four females and five males reared from infested pine tips collected by R. A. St. George in April, 1925. Some of the specimens are less contrastingly colored than the type.

Microbracon gemmaecola, new species.

In Muesebeck's key to the North American species of Microbracon[3] this species runs to couplet 65, where it agrees fairly well with the characters assigned to *argutator* (Say); but a comparison of specimens shows the antennae to be somewhat longer (very nearly as long as body in *gemmaecola*), with the subapical joints relatively longer. The head is somewhat thinner anteroposteriorly, and the hind tibia, except at the extreme base, and the tarsus are black.

Female.—Length 2.5 mm. Head hardly two-thirds as thick as broad, temples sloping; face, frons, and vertex laterad of ocelli finely shagreened, vertex behind ocelli and temples polished; malar space about three-fourths as long as first flagellar joint; space between eye and clypeal opening more than half as wide as opening; antennae 27 to 30 jointed (28 in holotype), very nearly as long as body, flagellum tapering slightly beyond middle, basal joints two-thirds as thick as long, subapical joints distinctly though not conspicuously longer than thick. Thorax smooth and polished; notauli with sparse hairs; mesoscutum posteriorly with scattered, coarse, shallow punctures; propodeum posteriorly faintly finely reticulated and with a stump of the median carina and radiating ridges; stigma slightly more than a third as broad as long, radius before middle, second abscissa of radius barely twice as long as first and more than half as long as third, with which it forms a slight angle, first intercubitus two-thirds as long as second abscissa of radius. Abdomen broadly oval, opaque, granularly punctate, second tergite as long as first and longer than third, more coarsely sculptured with an

[3] MUESEBECK, C. F. W. A REVISION OF THE PARASITIC WASPS OF THE GENUS MICROBRACON OCCURRING IN AMERICA NORTH OF MEXICO. U. S. Natl. Mus. Proc., v. 67, art. 8, 85 p., illus. 1925.

irregularly rugose area in basal middle, and medially emarginate at apex; ovipositor sheath barely as long as abdomen exclusive of first tergite, rather stout.

Pale yellowish ferruginous, with back of head, lateral lobes of mesoscutum, mesosternum, and propodeum stained with piceous; antennae, stemmaticum, tarsi, hind tibia except base, and apices of other tibiae black; wings hyaline, faintly infumate in basal half.

Male.—Runs to *argutator* in Muesebeck's key. Like female, but malar space much shorter; antennae longer and more slender, the subapical joints nearly twice as long as thick; second tergite less distinctly emarginate.

Type locality.—Nantucket, Massachusetts.

Host.—*Rhyacionia frustrana* (Comstock).

Type.—Cat. No. 40104, U.S.N.M.

Described from seven females and eight males reared during March, April, and May, 1926, by L. W. Bailey from host in pine tips at the type locality and two females and one male reared by the author from the same host at Falls Church, Va., May 17 to 21, 1925.

The individuals of the Nantucket series display comparatively little variation and that mostly in the extent of the piceous staining of head and thorax. The Virginia specimens lack this staining entirely.

EURYTOMA PARVA (GIRAULT) PHILLIPS AND ITS BIOL-OGY AS A PARASITE OF THE WHEAT JOINTWORM, HARMOLITA TRITICI (FITCH) [1]

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INTRODUCTION

The life history of *Eurytoma parva* (Girault) Phillips [3] indicates strongly that it is very gradually breaking away from the habit of parasitism upon animals and is becoming phytophagous. As pointed out by Gahan (1),[4] this is perhaps a recent specialization. There are strong indications that some individuals may even now develop upon plant tissue alone. However interesting this insect may be to the biologist, it is of no less interest to the layman, since at present it is one of the most important parasites of a very destructive pest of wheat, *Harmolita tritici* (Fitch).

In the light of recent observations it seems almost certain that at least the majority of the American species of Eurytoma found associated with various species of Harmolita have a life history parallel with that of *Eurytoma parva*. This assertion is not founded upon actual observa-tion but upon analogy. For example, *E. parva* begins to emerge at about the time that the peak of emergence of its host is reached. This time of emergence for a parasite is unusual, and yet it is true of all species of Eurytoma so far found associated with Harmolita. On the other hand, all of the other primary parasites of the joint-worms thus far observed emerge about a month later than their hosts. Secondary parasites may emerge at any time after the tem-perature has become sufficiently high for them to develop. Thus far there is no evidence that Eurytoma may be a secondary parasite on the jointworms.

The writer (6) figured the adult of *Eurytoma parva* and briefly mentioned this very unusual feeding habit in Farmers' Bulletin 1006, of the United States Department of Agriculture, published in 1918, giving the insect Girault's manuscript name, *parva*. Un-fortunately, Girault's paper was delayed in publication, thus making the writer responsible for the species.

In the present paper, not only are the facts relative to the biology of *Eurytoma parva* recorded in greater detail, but some of the struc-tural characters of the larva are described and compared with those of the host larva.

ORIGIN AND HISTORY

It is very difficult, if not well-nigh impossible, to determine definitely whether or not *Eurytoma parva* is a native American species, although perhaps the weight of evidence favors the negative.

The writer first began to study the habits of the jointworms in 1904. Infested cereal and forage plants were collected for a number of years over the greater part of the United States by various persons connected with the cereal and forage insect investigations of the Bureau of Entomology. During this time a great quantity of material was gathered, and many emerged adults were preserved for study. A number of species of Eurytoma associated with species of Harmolita from wild grasses were reared, but no record was found of the rearing of *E. parva* prior to 1913. Furthermore, there is no record in the bureau files of any species of Eurytoma having been reared from *Harmolita tritici* (Fitch), *H. vaginicola* (Doane), *H. grandis* (Riley), *H. secalis* (Fitch), *H. websteri* (Howard), or *H. hordei* (Harris) —these being the jointworms infesting the cereal crops—and this despite the fact that prior to 1913 jointworms from grains and grasses of eastern, central, and midwestern territory were being reared intensively. However, *E. pater* Girault was reared from jointworm-infested wheat stubble collected by the writer near Youngstown, Ohio, in 1911, according to bureau records. This is the first species of Eurytoma found associated with Harmolita from the small grains in America.

The first record of the occurrence of *Eurytoma parva* is dated June 4, 1913. The writer was then at Purdue University, La Fayette, Ind., where he was breeding various species of jointworms. While sweeping a field of wheat for *Harmolita grandis*, the writer captured a female of some species of Eurytoma. She was placed in a breeding cage on wheat that had been previously infested with *H. tritici*, and late in the same afternoon was observed to oviposit in the wheat stems. The following statement is made in the writer's notes of June 4, 1913: "I have never (to my knowledge) reared this from *H. tritici* and it will be an additional parasite for *tritici* should I succeed in rearing it." Four males were reared from this cage and have recently been identified by A. B. Gahan, of the Bureau of Entomology, as *Eurytoma parva*.

Although *Eurytoma parva* was first found in the Central States in 1913, in 1914 and 1915 it was much more plentiful in the Eastern States. Apparently it has spread gradually westward, as is well illustrated in Missouri. No specimens of *parva* had been reared from Missouri previous to 1917, so the writer and his associates introduced the species into one or two wheat fields of that State in 1918, 1919, and 1920. In 1920, however, *E. parva* was reared sparingly from collections made at localities in Missouri where the species had not been liberated. As a small number were introduced into that State at one or two points only, it seems safe to assume that at that time *E. parva* was just spreading into Missouri, independently of the introductions.

It seems strange that *Eurytoma parva* should not have been found in the collections made before 1913, since extensive collections and rearings of jointworms from the small grains had been made for nine years previous to that date. These rearings were from collections

made in practically every State where *Harmolita tritici* was known to be present. Moreover, the jointworms occurring in the small grains have been reared in America for many years, a fact which leads the writer to think that this parasite is possibly an introduced species.

The writer has made strenuous efforts to obtain from Europe named species of the genus Harmolita and some of their parasites, for comparison with American forms, but has been unsuccessful. However, Rimsky-Korsakov's (*8, p. 36–40*) descriptions and illustrations of adults and larvae leave little doubt that his (*Isosoma*) *Harmolita inquilinum* is a species of Eurytoma. Furthermore, its habits are similar to those of *E. parva*. It is singular that Hedicke (*2*), in his extensive work on the jointworms of Europe, fails to mention Rimsky-Korsakov's recently described species, or the latter's paper in which he records the parasitic and phytophagous habits of (*Isosoma*) *H. inquilinum*. Hedicke records only one American species, *H. hordei*.

CLASSIFICATION OF THE LARVAE OF EURYTOMA PARVA AND
HARMOLITA TRITICI

The most recent and comprehensive work on chalcidoid larvae is by H. L. Parker (*4*). In this paper Parker divides the larvae into seven main groups, based largely upon the external structural characters of the first instar. *Eurytoma parva* apparently more nearly fits into Group VI, there being some few points of disagreement. Parker evidently made a slight error in drawing up his description of Group VI, for on page 333 of his work he states that the thoracic segments bear three pairs of long sensory hairs and that the abdominal segments bear four pairs, while on page 284 he states that the thoracic segments bear six and the abdominal bear four sensory hairs, which agrees with the figures. The first instar of *E. parva* has the same number of sensory hairs on both the thoracic and abdominal segments. There is a very indistinct band of most minute spicules on the body segments, and there appear to be five pairs of open spiracles, though the second thoracic spiracle is very small.

The larva of *Harmolita tritici* does not seem to fit into any of Parker's groups. The first-instar larva comes nearer to Group II, perhaps, than any other, though it has many points of difference from all the groups. For example, the head is not broader than the body, and is very feebly chitinized; there are apparently no antennae or sensoria except in the buccal region; apparently no open spiracles; no bands of spicules around the segments; and the mandibles are simple and triangular. Therefore the larva of *H. tritici* might well constitute another group, making a total of eight groups according to Parker's classification, the new one having the following characters: Cylindrical in form, tapering toward each extremity; 13 fairly distinct segments, not including the head; head about the size of the thorax, conical in outline, the mouth being located decidedly subterminally near the midline of the ventral surface; apparently no antennae, spicules, sensory hairs, or open spiracles; mandibles simple and triangular in outline.

In locating the body setae or sensory hairs of both *Eurytoma parva* and *Harmolita tritici*, the writer has decided to mention and locate only those for one-half of the body throughout its entire length. It will perhaps be less confusing to locate all the setae on

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the head, and this has been done, though all lateral views of the larvae show only half the total number of setae. This should be borne in mind in reading the descriptions.

DESCRIPTION

HARMOLITA TRITICI (FITCH)

First Instar

Cylindrical in form, tapering toward each extremity; 13 fairly distinct segments in addition to the head; head about size of thorax, conical in outline, the mouth located decidedly subterminally near midline of ventral surface; apparently no antennae, spicules, sensory hairs, or open spiracles; two pairs of sensoria in maxillary region; mandibles simple and triangular in outline, whitish, and very feebly chitinized. Entire larva a translucent whitish color and very soft (fig. 1, B).

Average length of three individuals 0.74758 mm., average width 0.128565 mm. These measurements were taken from living specimens.

Full-Grown Larva

Cylindrical in form, tapering slightly toward each extremity, and having 13 segments, not including the head, which is almost hemispherical (fig. 1, A). Mouth parts situated approximately in middle of face or front of head. Head narrower than body segments. Antennae broader than long and scarcely visible without high magnification. Eight small sensory setae on the head, all located laterally except one pair, which is situated at the base of the labrum (fig. 1, A; fig. 2, A). The two most dorsal setae are about twice the length of any of the others. Labrum rather large and convex, with three fairly distinct lobes, the central lobe being the largest. Eight minute sensoria on labrum, a group of three at each side of mouth, and a pair on central lobe of labrum.

The ventral mouth parts form the entire lower part of the head; they are very convex, slightly lobed at the mouth, and bear 14 minute sensoria, 7 to each lateral lobe.

Mandibles simple, triangular, rather heavily chitinized, and brownish.

Thoracic segments somewhat longer than the abdominal; first thoracic distinctly the longest segment of the body, and bearing five very inconspicuous sensory hairs, there being one upper and one lower tergal, one lateral, one sublateral, and one sternal; second and third segments each bearing four sensory hairs, the upper tergal hair being absent; each of the remaining body segments bearing two sensory hairs, one tergal and one lateral, except the anal segment, which bears three. All sensory setae of the body very small and inconspicuous and arranged as indicated in Figure 1, A.

Average length of eight individuals 3.8942 mm., average width 0.8942 mm. The measurements averaged were taken from larvae fixed in Carnoy's fluid.

EURYTOMA PARVA (GIRAULT) PHILLIPS

Egg

The egg is ovoid with a long pedicel at one pole and a short pointed pedicel at the other (fig. 1, G). The length of the long pedicel is about one and a half times that of the egg. Eggs freshly dissected from the abdomen of a female are white; after oviposition they are of a dirty brownish color.

The average length of three eggs, including the pedicels, was found to be 1.0378 mm., and the average width 0.1515 mm. The measurements averaged were taken from specimens preserved in Stromsten's fluid.

First Instar

According to Parker's description, the shape of the first-instar larva is typical of the genus Eurytoma (fig. 1, E, F). Larva translucent, whitish in color, its segmentation very distinct; head brownish; head and body bearing numerous sensory hairs of different lengths.
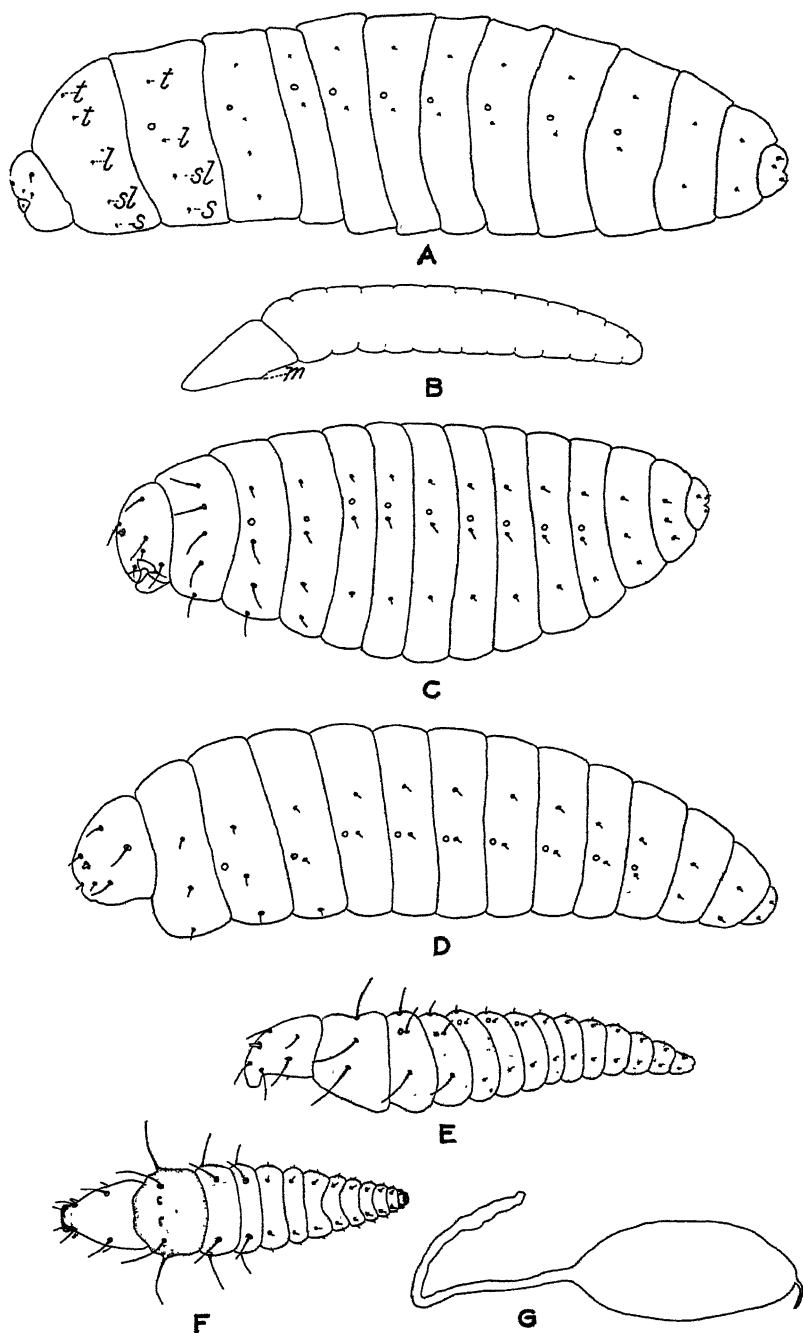
FIG. 1.—A, lateral view of full-grown larva of *Harmolita tritici*, showing position of sensory hairs: *t*, Tergal sensory hair; *l*, lateral sensory hair; *sl*, sublateral sensory hair; *s*, sternal sensory hair. B, lateral view of first-instar larva of *H. tritici*; *m*, mouth. C, lateral view of full-grown larva of *Eurytoma parva*, showing position of sensory hairs. D, lateral view of second-instar larva of *E. parva*, showing position of sensory hairs. E, latero-ventral view of first-instar larva of *E. parva*, showing position of sensory hairs. F, ventral view of first-instar larva of *E. parva*, showing chitinized plate of first thoracic segment, somewhat dorsally flexed. G, egg of *E. parva*. A and C are drawn to the same scale; B, D, E, F, and G are drawn to a uniform scale, but are more highly magnified than A and C

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Average length of five living larvae 0.7880 mm., average width 0.1884 mm.

Outline of head (fig. 1, E, F) reminds one somewhat of the beak of a vulture, approximately as wide as thick, but distinctly longer than wide, brownish in color, and strongly chitinized; two antennae, approximately three times as long as broad, but with only one segment; head bearing 10 prominent sensory hairs, of which 4 are on the ventral surface, a pair just above the labrum, a pair just above the antennae, and 1 hair on each lateral face (fig. 1, E). Mouth situated on ventral side, at apex of the somewhat elbow-shaped head, and surrounded by a heavily chitinized ring; mandibles sickle-shaped, strongly chitinized and almost black, the bases brownish; labrum with six very indistinct sensory organs; sensory organs of labium so minute that the writer could not identify them with certainty. Parker (*4*) states that *Eurytoma dentata* Mayr has a pair of sensory organs below the mouth, but does not place them. He does not mention any sensory organs for *Eurytoma rosae* Nees.

Body composed of 13 segments, the thoracic segments being the longest, and the remaining segments tapering sharply posteriorly. Each thoracic and each abdominal segment bearing three sensory hairs; those of the first thoracic segment long and coarse, those on the remaining segments gradually diminishing in size and length until those of the last two or three segments are almost invisible. These sensory hairs are arranged in three rows (a lateral row near the spiracles, a tergal row, and a sternal row), each extending the full length of the body. There is more or less of a band of very minute spicules around each segment, visible only with high magnification. Five spiracles, all apparently open.

Ventral surface of first thoracic segment shaped somewhat like the sole of a square-toed shoe, strongly chitinized, and having a pair of chitinous processes near center (fig. 1, F).

When the larva is ready to molt, the head is much smaller than the thoracic segments, all the body segments being much more robust.

SECOND INSTAR

In the first molt the larva undergoes a great change in appearance. The second instar resembles neither the first instar nor the full-grown larva, though it is more like the latter than the former. The larvae appear to be more delicate while in this instar than at any other time.

Head somewhat intermediate in shape between that of first instar and that of full-grown larva (fig. 1, D). Head capsule very feebly chitinized; first thoracic segment extending far below the ventral surface of the head; head whitish and bearing 12 sensory hairs, of which 8 are large and 4 small; 4 of the large hairs are on the cephalic aspect of the head, just above the antennae, 1 is on each lateral face, and 1 laterad of each mandible; of the small sensory hairs 2 are just above the labrum, and 1 laterad of the base of each mandible; antennae about twice as long as wide; mouth located on apex of head; mandibles triangular in outline, very feebly chitinized, and bearing a single denticle; sensory organs of labium appearing so inconspicuous and indistinct that the writer can not enumerate them with accuracy; each thoracic segment bears 3 sensory hairs and the abdominal segments 2 each, arranged as indicated in Figure 1, D; all sensory hairs except those on the last two or three abdominal segments much smaller than those in any other instar. Nine pairs of open spiracles.

Average length of six individuals (living specimens) 1.4147 mm., average width 0.4096 mm.

The writer has definitely identified as many as three instars, the first, the second, and the full-grown larva. After the second instar the larvae so closely resemble the full-grown larva that it has been impossible to identify the third and fourth with certainty. There are, however, apparently four molts, making five instars.

FULL-GROWN LARVA

Full-grown larva somewhat barrel-shaped, distinctly broader in midregion and tapering rather sharply toward each extremity. Body composed of 13 segments, not including the head; head almost hemispherical, narrower than the body segments, and translucent-whitish except in mandibular region, where it is brownish; mouth parts pointing downward; antennae distinctly longer than broad; on the head 12 sensory setae, 8 large and 4 small, the latter situated just above bases of mandibles; of the large setae 4 are on the dorso-cephalic

aspect of the head, above the antennae, one on each side of head, near center of lateral face, and one caudad of base of each mandible (fig. 1, C, and fig. 2, D). Labrum three-lobed and bearing eight minute sensoria, there being a group of three near each corner of mouth and a pair on central lobe; the ventral mouth parts form, together, a fused region, which anteriorly is trilobed, corresponding to the component median labial, and the two lateral maxillary regions. This fused region (fig. 2, D) bears 16 sensory hairs and organs, 8 on each lateral lobe or maxillary region; each lateral lobe bears one comparatively large circular structure, which is possibly a vestigial maxillary palp; it is rather strongly chitinized, convex, and extends above the surrounding surface of the lobe or maxillary region; each maxillary region has 4 rather prominent sensory hairs, or setae, and 3 inconspicuous sensory hairs, or papillae. (See fig. 2, D.) In the legends to the illustrations these have been called setae and papillae, respectively. The mandibles (fig. 2, G, H) are heavily chitinized, almost black, triangular in outline, and each has one large denticle on the inner margin. The position of the mandibles and their articulation to the different parts of the mouth frame are shown in Figure 2, F. For an interpretation of these parts the legend of Figure 2 may be consulted. The thoracic segments gradually increase in size posteriorly; the first thoracic segment is the longest and the third abdominal segment the thickest body segment. The first thoracic segment bears 5 prominent sensory hairs, the second and third 4 each, and the abdominal segments 3 each; these abdominal sensory hairs are arranged laterally in three longitudinal rows (fig. 1, C). The sensory hairs of the first and second thoracic segments are conspicuously longer and larger than those of the remaining segments of the body. There are nine open spiracles.

Average length of 100 living specimens 2.7330 mm., average width 1.1752 mm.

SEASONAL HISTORY

In the vicinity of Charlottesville and Warrenton, Va., the adults of *Eurytoma parva* emerge from about the beginning to the middle of May, the time depending upon weather conditions. Apparently there is a preoviposition period of several days, though, since the species does not breed very freely in confinement, this point is rather difficult to determine.

Adults lived in confinement for nearly a month, a much longer time than jointworm adults live under similar circumstances. This comparatively long life, if we assume that it normally obtains under field conditions, greatly increases the opportunity of the females to find suitable places for oviposition.

The eggs hatch in about four or five days, the larva rupturing the eggshell at the base of the long pedicel. The larva apparently passes through four molts, though this fact could not be definitely determined, and becomes full grown in about 24 days. It then remains in the larval stage in its cell in the wheat stubble until the following spring. Excrement is voided in the cell after the larva becomes full grown, and usually just before the prepupal stage. This unusual habit has been noted by the writer and Poos (*7, p. 413, 419, 425*) in a previous paper on the jointworm parasites, and by others in the case of Donacia, Myrmeleo, and the rice weevil. In the three cases last named the excrement when voided is used in building up the cocoon and is apparently reserved for this purpose. In the case of *Eurytoma parva* and other jointworm parasites no cocoon is formed. The food of these small Hymenoptera perhaps contains very little matter that is not entirely digested and absorbed, and hence no fecal matter is elaborated. Besides, if excrement should be voided at regular intervals, their food supply might be contaminated, and this perhaps might lead to complications arising out of bacterial growth. Some time in the latter half of March the larvae change to pupae, and the adults emerge in the first half of May.
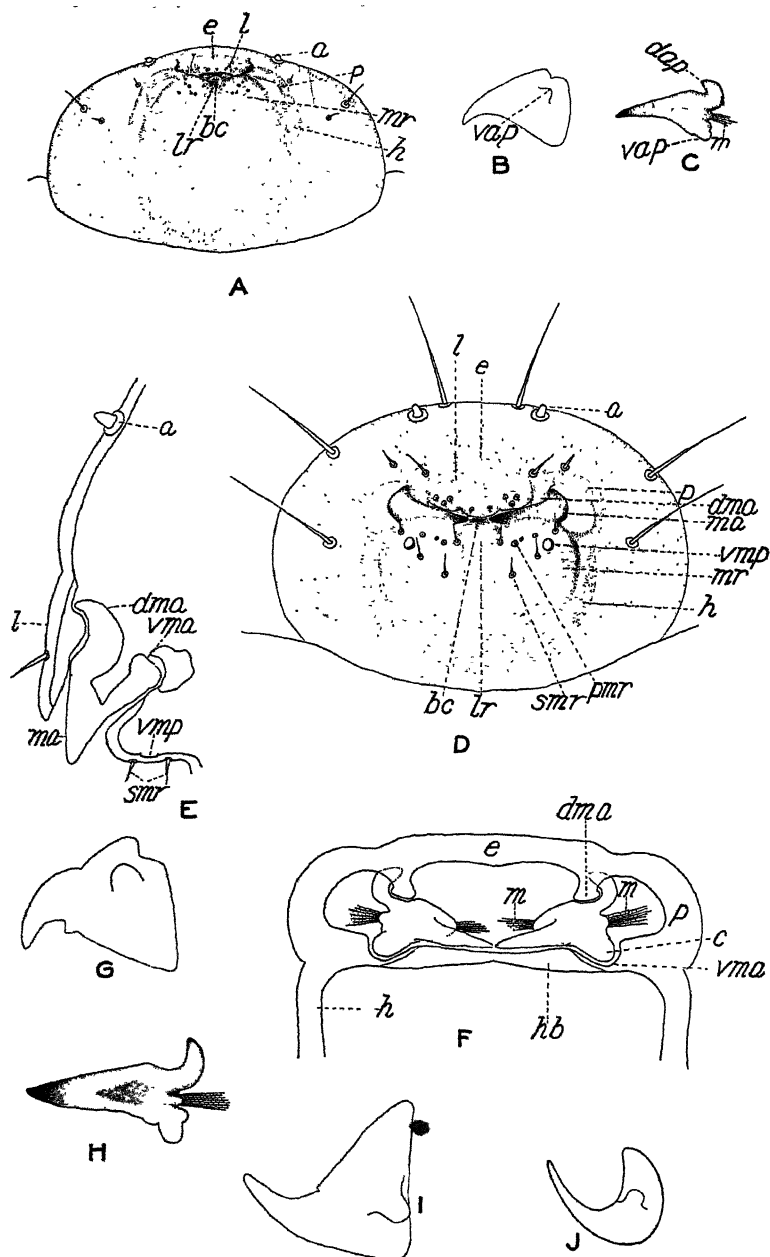
FIG. 2.—A, ventral view of head of full-grown larva of *Harmolita tritici*, showing position of
mouth parts; B, ventral view of mandible, showing condylar process; C, exterior-lateral view,
showing dorsal and ventral articular processes; D, ventral view of head of full-grown larva
of *Eurytoma parva*, showing position of mouth parts; E, longitudinal section through maxil-
lary region, showing labrum, mandibular articulations, what has provisionally been termed
the vestigial maxillary palp, and two adjacent sensory hairs of maxillary region (the cuticle
in this region had shrunk in such a manner as to involve the "vestigial maxillary palp" and
three sensory setae in the same plane with the antenna); F, mouth frame of full-grown larva
of *E. parva;* G, ventral view of mandible of full-grown larva of *E. parva*, showing condylar
or ventral articular process and denticle on inner margin of mandible; H, exterio-lateral
view of same, showing dorsal and ventral (condyle) articular processes and muscular attach-
ment; I, ventral view of mandible of second instar of *E. parva;* J, ventral view of mandible
of first instar of *E. parva*. (For explanatory legend see p. 751.)

(750)

The records on individual farms at Warrenton, Va., since 1916 show that *Eurytoma parva* has gradually increased in numbers at the expense of *Harmolita tritici* until at present it is one of the most important parasites of the wheat jointworm. The bureau records are not so complete for other parts of the country, but the indications are that in the Central States the trend is in the same direction.

NATURAL ENEMIES

Eurytoma parva, like *Harmolita tritici*, suffers from the attacks of parasitic enemies, though not to the same extent. The lower susceptibility of the former to attack by enemies appears to be due to the fact that the full-grown larva is very active, whereas the larvae of *H. tritici* are very sluggish. The least disturbance will cause a larva of *E. parva* to wriggle vigorously. Undoubtedly by their activity these larvae crush many enemy larvae of the first instar that attempt to attack them. This has actually been observed in some instances.

Four parasites have been found attacking larvae of *Eurytoma parva—Digfopinotus aureoviridis* Crawford, *Eupelminus saltator* (Lind.), *Eupelmus allynii* (French), and *Homoporus chalcidiphagus* (Walsh and Riley). The last named would probably be only occasionally successful in its attacks; its larva is very sluggish and while in the first instar would very easily be crushed by the very active larva of *E. parva*.

BREEDING METHODS

The usual type of flowerpot breeding cage with a glass cylinder for a cover was used for the majority of observations in 1924, at Charlottesville, Va., and fairly good results were obtained; in fact, a greater degree of success was achieved than at any previous time, perhaps because of a special method of handling the adults. As they emerged they were transferred to glass cylinders about 1½ inches in diameter and about 10 inches long. One end of each cylinder was covered with cheesecloth and the other plugged loosely with absorbent cotton. A little melted beeswax was poured on the inside of the cylinder and smoothed out flat, and a droplet of 17 per cent (approximately) sugar solution was placed on the beeswax for the adults to feed on. The beeswax kept the sugar solution from spreading over the side of the glass cylinder, where it would entangle the parasites. These stock cages were placed in a cool, dark place; the adults could in this manner be kept alive for two or three weeks, as a reserve supply from which to replenish the breeding cages. Previously the writer had been unable to maintain a reserve of the adults until the plants infested with the jointworm were in proper condition for the parasites to oviposit.

EXPLANATORY LEGEND FOR FIG. 2

A and D are drawn to the same scale; B, C, E, F, G, and H are drawn to a common scale, but are more highly magnified than A and D; I and J are drawn to a common scale, but are much more highly magnified than the others.

Explanations of symbols: *a*, Antenna; *bc*, buccal cavity, *c*, condyle; *dap*, dorsal articular process of mandible; *dma*, dorsal mandibular articulation; *e*, epistoma; *h*, hypostoma; *hb*, hypopharyngeal bracon; *l*, labrum; *lr*, labial region; *m*, muscle; *ma*, mandible; *mr*, maxillary region; *p*, peristoma; *pmr*, papillae of the maxillary region; *smr*, setae of the maxillary region; *vap*, ventral articular process or condyle; *vma*, ventral mandibular articulation; *vmp*, "vestigial maxillary palp."

All who breed small Hymenoptera know that closely related forms are often difficult to recognize and separate while alive, particularly if there are a number of individuals in the same breeding jar. For different breeding experiments it often becomes necessary to separate these living forms with certainty—no small matter, if several species and many specimens of each are involved. Eight or ten years ago the writer hit upon a very workable plan which has admirably met this difficulty. It may be known to some workers, and of interest to others.

These small Hymenoptera are collected from the breeding jar and placed indiscriminately in small vials. The plugged end of the vial is turned downward for a few minutes, whereupon the insects collect in the upper end. The plug is then quickly removed, the mouth of the vial held over a small cake of ice, and the end of the vial is given two or three vigorous taps with the fingers. The insects fall on the cake of ice, and all are immediately benumbed. The ice is then placed in a shallow saucer in which the specimens may be examined under a microscope and the species separated. The ice used should be nearly flat on both the upper and lower surfaces, so that the insects will not slide off.

When the specimens are removed from the ice those of each species should be placed in a vial with strips of absorbent paper, and in a few minutes they will become dry and be as active and alert as ever, apparently none the worse for the experience. In this way the vials may be stocked with the required number of individuals of each sex. When they have become dry they are ready to be transferred to the breeding cages.

Besides the method here described, involving the usual flowerpot breeding cage, other methods were tried for the purpose of demonstrating particular features of the life history of *Eurytoma parva.* Three methods of rearing larvae of *E. parva* in cell slides were tried, all resulting in failure, though 16 slides were used in each experiment. The cell slides were made by grinding with a dentist's burr a cavity 5 mm. long, 2 mm. wide, and 1.5 mm. deep, in a piece of common glass about two-thirds the size of an ordinary glass slide. A first-instar larva of *E. parva* was dissected from a cell of *Harmolita tritici* and placed in a cell slide on a full-grown larve of *H. tritici,* and a cover glass was partly sealed down over the cell with a droplet of honey. Another method was simply to place the cover glass over the cell and then put the slide in a moist chamber. A third method was to place the cover partly over the cell and put the slide in a moist chamber. All of the slides were kept in darkness in a desk drawer.

Two methods involving transfer were tried, one a failure, the other but slightly successful. Suitable plants in breeding cages (pot cages) were selected and a cell of *Harmolita tritici* containing a full-grown larva was taken from one and carefully opened under a binocular microscope. A larva of the first or second instar of *Eurytoma parva* was carefully removed from its cell and placed on the full-grown larva of *H. tritici* in the cell just previously opened. The cell was then closed very carefully and wrapped with the end of a green leaf from the plant from which the larva of *H. tritici* was taken. It was hoped that the green leaf would prevent evaporation and make conditions more nearly normal.

By the second transfer method a tender wheat stem was located in one of the pot cages that was infested with *Harmolita tritici*, and a tender gall then located in which the host larva had but recently hatched. The cell was carefully opened with a fine needle, under a binocular microscope, the host larva removed, and in its place was inserted a first-instar larva of *Eurytoma parva* that had not previously fed on the larvae of *H. tritici*. The cell was then carefully closed and wrapped in a green leaf, as in the method described in the preceding paragraph.

Ten trials were made of the first transfer method, only one of which was successful. In this case a larva of the second instar was placed on a full-grown host larva. The parasite larva was removed from a cell in which it had evidently devoured a young host larva and had then begun to feed on the sap of the plant. This parasite larva completed its development upon the larva of *Harmolita tritici* and evidently was unable to secure plant sap, as the appearance of the plant tissue where the host larva was confined indicated that the tissue dried up soon after the transfer was made. The only striking thing about this parasite larva was the fact that it was slightly below normal in size.

Nine trials, all failures, were made of the second transfer method. The results, however, do not have the ordinary value of negative results, because in no case did the plant tissue appear to heal after the operation; in every instance it died and dried out. Plainly, there was absolutely no way for the young larvae to obtain food from the plant tissue.

The situation may be summed up as follows:

1. *Harmolita tritici* must oviposit in a wheat stem before a female *Eurytoma parva* will deposit eggs in the stem, although *E. parva* places a great many eggs outside the jointworm cells.

2. When an egg of *Eurytoma parva* hatches just outside a jointworm cell and there is no host larva in its immediate vicinity it proceeds at once to feed on the sap of the plant. The inference is, therefore, that it may reach maturity on plant sap alone.

3. Should a larva of *Eurytoma parva* find itself upon a mature larva of *Harmolita tritici* it is possible for the former to reach maturity by feeding upon this mature larva instead of upon plant tissue. Such cases would ordinarily be rare under field conditions, since larvae of *H. tritici* are usually in about the first or second instar when the larvae of *E. parva* hatch, and these small larvae do not supply food enough to carry the *E. parva* to maturity.

4. In the case of perhaps most frequent occurrence, the larva of *Eurytoma parva* while in its first instar feeds on the larva of *Harmolita tritici*, and then changes to a plant diet.

It will be seen that the larva of *Eurytoma parva* is at present in a highly plastic or adaptive condition, a fact which indicates to the writer that it is in the process of changing over from parasitism upon animals to phytophagy, while as yet entirely dependent upon *Harmolita tritici* for the stimulus leading to oviposition.

COMPARISON WITH THE HOST LARVA

The most striking differences between the larvae of *Harmolita tritici* and those of *Eurytoma parva* occur in the first instar (fig. 1, B, E, F). The larva of *H. tritici* is apparently a most delicate

organism, and capable of only slight movement. Its mandibles are extremely minute and feebly chitinized; setae, if present, are too small or inconspicuous to be seen under a magnification of 400 diameters; no open spiracles are apparent, the body segments are barely indicated, and all are thinly membranous. In contrast to this larva the first-instar larva of *E. parva* (fig. 1, E, F) is a rugged, motile organism. The mandibles are stout, large, highly chitinized, and dark brown in color; the setae are numerous, long, and stout; there are five pairs of open spiracles; the body segments are well defined, and the entire ventral-surface of the first thoracic segment appears to be heavily chitinized.

As previously stated, the differences in the remaining instars are much less striking, but the larva of *Eurytoma parva* is much more active, and the setae and mouth parts more strongly developed. The shape of the larvae in the two species is characteristically different throughout. The full-grown larva of *E. parva* is very active, and that of *Harmolita tritici* sluggish; the two are quite different in shape (fig. 1, A, C); the mouth parts of *E. parva* are more prominent and more highly chitinized; the setae are more numerous and much larger and stouter; the color of both larvae is almost a canary yellow.

The larger and more highly chitinized mandibles serve the full-grown larva of *Eurytoma parva* to very good advantage. During its last instar the wheat stems are much more woody, and there does not appear to be such a generous flow of plant sap. This larva is therefore obliged to shred and lacerate the walls of its cell more vigorously in order to maintain a good flow of sap; this fact will account for the large amount of frass in the cells, as already mentioned by Rimsky-Korsakov (*8, p. 19–20, 36–40*) in the case of *Harmolita inquilinum*. This writer, however, apparently failed to interpret correctly the connection between the accumulated frass, the stronger mandibles, and the mode of life of the parasite. The larvae of *H. tritici* mature earlier, while the wheat plants are more succulent, the bodies of the larvae being almost constantly bathed in the rich plant sap. They need then to lacerate the plant tissue but slightly to maintain the flow of sap, and naturally less frass is accumulated.

This feature is more strongly emphasized in *Harmolita grandis* (Riley), *H. websteri* (Howard), and *H. albomaculata* (Ashm.). In fact, all species of Harmolita that develop within the center of the stem, where they are not surrounded by a plentiful flow of sap such as surrounds those which, like *H. tritici*, develop in the tissues of the stem walls, are forced to tear and lacerate more extensively the walls of the stem in order to maintain a constant supply of sap. As a result, a greater amount of frass is always collected under these conditions.

The writer has not had an opportunity to make a detailed study of the internal anatomy of either *Harmolita tritici* or *Eurytoma parva*, but from a casual examination of stained sections there appears to be a noticeable difference in the development of the salivary glands of the first and perhaps the second instars of the two species. These glands are noticeably larger, and the gland cells themselves are larger, in the vegetable feeder *H. tritici* than in *E. parva*, which, in these instars, is parasitic upon animal tissue. In the full-grown larvae, however, which in both species are vegetable feeders, the glands are more nearly of the same size.

INJURY TO THE PLANT

To speak of plant injury in discussing the biology of an insect parasite sounds a little odd, perhaps, but the question may properly be asked whether *Eurytoma parva* is a parasite or whether it should not be classed as a plant pest. The writer does not hesitate to class it as a parasite. The wheat plant is damaged perhaps more severely by the larvae of this insect when they are once established within it than by the larvae of *Harmolita tritici;* but the point is that the wheat plant would not be attacked by *E. parva* at all if larvae of *H. tritici* were not present in the stem walls. All larvae of *H. tritici* with which a larva of *E. parva* comes in contact are destroyed; the numbers of the host are therefore reduced and actual damage to wheat, on the part of *H. tritici,* is thus curtailed.

The larvae of *Eurytoma parva* deform the wheat stalks and deplete the nourishment in the same manner as do those of *Harmolita tritici,* and perhaps inflict slightly greater injury, since they feed a little longer and are obliged to shred the tissue more extensively in order to maintain a flow of sap. Externally, the conformation of the individual cells and the general aspect of the distorted wheat stem are so similar in the case of both insects as to render identification from these characters impossible. The cells of *E. parva* are often somewhat darker in color than those of *H. tritici,* but this character is not always reliable.

PARTHENOGENESIS

Two breeding cages were used in 1924 to test parthenogenesis on the part of *Eurytoma parva.* Only virgin females of *E. parva* were placed in the cages; they oviposited about as freely as those in the other cages, and the act of oviposition seemed normal. All the cells of the jointworm galls in these two cages were carefully opened April 9, 1925, and a few unemerged adults of *Harmolita tritici* were found, the remaining cells containing dead larvae which could not be identified. This evidence is inconclusive, though the fact that the virgin females appeared to oviposit normally and freely, and the further fact that nearly related species have proved to be parthenogenetic, make it seem probable that *E. parva* is also parthenogenetic.

PARASITIC AND PHYTOPHAGOUS HABITS OF THE LARVA

So far as the writer has been able to learn, only two cases of feeding habits such as those here described have been observed by other investigators on the Chalcidoidea. Nielsen (*3, p. 46*), in Denmark, made a very brief statement to the effect that he found an undetermined species of Eurytoma that first destroyed the young larvae of *Cryptocampus angustus* Htg., which lived in the heartwood of willow twigs, and afterwards fed and completed its development in the pith of the willow stems. Phillips simply mentioned this feeding habit for *E. pater* Girault in 1917 (*5, p. 145*), and for *E. parva* in 1918 (*6*). In 1914 Rimsky-Korsakov (*8, p. 19-20, 36-40*) gave an excellent account of similar habits of (*Isosoma*) *H. inquilinum* (Rimsky-Korsakov) in rye, although there are in this connection a number of interesting points that he was unable to clear up.

From Rimsky-Korsakov's description of the adult of (*Isosoma*) *Harmolita inquilinum*, and from the illustrations of the adults and the larva, the writer feels sure that American systematists would place it as a Eurytoma. The host, *H. rossicum* (Rimsky-Korsakov), develops in rye stems. The infested sections of rye stalks bearing the galls are illustrated in Figures 19 and 20, on pages 32 and 33, respectively, of the work cited. Some of these galls resemble very closely the galls formed by the American species *H. secalis* (Fitch), while the galls on other sections resemble very closely those of the American species *H. vaginicola* (Doane) on wheat. The illustrations of *H. rossicum* are typical of American *Harmolita*.

Ever since 1917, when it was learned that the larvae of Eurytoma are both parasitic and phytophagous, the writer has been deeply interested in the species of Eurytoma that are associated with Harmolita. Difficulty in securing identification of the adults, failure to breed them freely in confinement, and their apparently slight economic importance somewhat discouraged further investigations at that time. In recent years *E. parva* has taken first rank as a parasite of the wheat jointworm, systematists have become willing occasionally to risk identification of material, and a greater measure of success has been obtained in rearing specimens under observation.

The majority of the species of Harmolita observed will oviposit freely in confinement without taking food, and oviposition begins soon after emergence; while *Eurytoma parva* apparently has a much longer preoviposition period and requires food. By keeping the adults confined in vials plugged with cotton, supplying them with sirup containing 17 per cent of sugar before placing them in breeding cages, and feeding them while in those cages, a fair measure of success was obtained.

A number of females of *Eurytoma parva* were dissected, and in no case were more than two or three mature eggs found in an abdomen, even in newly emerged specimens dissected before they had an opportunity to oviposit. Adults of the wheat jointworm have from 50 to 100 eggs in the body cavity before oviposition begins.

Females of *Eurytoma parva* refuse to oviposit in stems in which *Harmolita tritici* has not previously deposited eggs. Furthermore, a large number of dissections of field and cage material indicate that the females of *E. parva* prefer to oviposit in cells of *H. tritici* containing a larva of the first or second instar, as it is very rare to find larvae of *E. parva* on larger larvae of *H. tritici*; eggs and young larvae of *E. parva* were practically always found upon larvae of the first or second instar, even though older larvae of *H. tritici* were present in the stem. This condition is easily brought about both in the field and in the breeding cages, since the adults of *H. tritici* emerge in advance of those of *E. parva*, and since the adults of *E. parva* live much longer. The latter have been kept alive for about a month, while a week or 10 days is about the limit of adults of *H. tritici*.

The female of *Eurytoma parva* may deposit from one to six eggs in the immediate vicinity of a larva of *Harmolita tritici*, the eggs often being placed outside the jointworm cell. Upon hatching, a larva that finds itself outside a cell of *H. tritici* does not seem to seek out a

larva of *H. tritici*, but begins at once to feed on the sap of the plant.
This fact can easily be ascertained, as the larva is almost transparent
and the chlorophyll appears very distinctly in the digestive tract.
Numerous larvae apparently nearly ready to molt have been found
that had not entered a cell of *H. tritici*, and had never fed on any-
thing but plant sap. In such cases there was always a distinct little
pocket in which the larva was resting and feeding. This observation
certainly indicates that the larvae can, and in many instances possibly
do, reach maturity, and during their existence feed on nothing but
plant sap. These observations were made from a great number of
dissections of both cage and field material.

Probably the majority of the eggs are placed in the jointworm cells,
but rarely in direct contact with the larva of *Harmolita tritici*. Upon
hatching, the larval parasites devour the host larva in a short time.
They have often been found when they had just completed feeding
on the Harmolita larva, and were still in the first instar and not ready
to molt. In such cases they apparently proceed at once to feed on
the plant sap. They seem to be able to migrate through the plant
tissue for microscopical distances only.

Upon hatching, many larvae of *Eurytoma parva* find themselves in
the cell with a larva of *Harmolita tritici* of the second instar, and
proceed at once to devour it. The majority, perhaps, are placed on
host larvae of the first instar. As previously stated, the adult female
of *Eurytoma parva* seems to prefer the jointworm larvae in the first
and second instars for oviposition, even though host larvae of the later
instars are available.

The larvae of *Eurytoma parva* simply tear a hole in the body wall
of the host larva with their mandibles, and suck out the contents
of the body. After having apparently consumed the host larva
they continue to occupy the jointworm cell and begin to feed on
the plant sap. The evidence that the larvae of *E. parva* do feed
upon the larvae of *Harmolita tritici*, and do not simply kill them in
order to gain possession of the cell, is the fact that in all such
cases no chlorophyll is seen in their bodies until after the host larva
is consumed.

During this study approximately a thousand cells of *Harmolita
tritici* have been opened which contained larvae of *Eurytoma parva*
or had them in the immediate vicinity, and in only a few instances
were the Eurytoma larvae found feeding on the larvae of *H. tritici*
of the third or fourth instar. In the few exceptions two jointworm
cells were very close together, with a very thin partition wall. The
larva of *E. parva* apparently breaks accidentally through this wall
in lacerating the plant tissue to secure more plant sap. In these
instances the second jointworm larvae are killed and perhaps de-
voured, though this point has not been definitely established.

If several larvae of *Eurytoma parva* hatch in a single jointworm
cell, a struggle ensues and the strongest individual apparently sur-
vives and takes possession of the cell. This is indicated by the
occasional finding of several partially destroyed first-instar larvae of
E. parva in a cell of *Harmolita tritici* with a strong, vigorous-looking
individual of the same instar.

SUMMARY

At present *Eurytoma parva* is apparently one of the most important parasites of the wheat jointworm, *Harmolita tritici*.

It is both parasitic and phytophagous in habit. This is the first detailed observation made of this relationship for American Chalcidoidea.

Evidence has been produced which indicates that *Eurytoma parva* may reach maturity on plant sap alone.

In one instance a larva of the second instar of *Eurytoma parva* that had evidently consumed a larva of *Harmolita tritici* and had begun to feed on plant sap was reared to maturity upon a full-grown larva of *H. tritici*.

The observations detailed in this paper indicate that *Eurytoma parva* is gradually changing from a parasitic to a phytophagous habit.

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THE RATE OF PASSAGE OF FOOD THROUGH THE DIGESTIVE TRACT OF THE HEN [1]

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REVIEW OF LITERATURE

The earliest recorded attempt to determine the time required for food to pass through the gastrointestinal tract of hens or other birds is probably that of Weiske, reported in 1878 (*9*).[2] After Weiske had fed 2 geese on dandelion leaves for 8 days, he abruptly changed the ration to barley grains, and within 3 hours and 25 minutes he found the barley grains in the excrement of the birds, swollen but otherwise little altered in appearance. Within 3 hours more, that is, about 6½ hours after the feed was changed, he observed that the excrement consisted entirely of residues of the barley with no green feed. After the geese had been fed grain only for several days, this ration was discontinued and green feed only was given. The green feed first appeared in the feces of one bird after 1 hour and 40 minutes and in that of the other after 1 hour and 45 minutes. After 3 hours and 30 minutes the excrement of both birds consisted almost exclusively of residues of the green feed, with only scattered bits of the barley kernels.

Recently a few rather extended efforts have been made to gather digestion data on poultry by methods similar to those used with human beings. T. G. Browne (*2*) determined the time required for food to traverse the tract of hens by a study of the droppings. In his early experiments he used small colored seeds, such as those of red millet and clover, but these he found unsuitable because of the difficulty of identifying them in the feces. Bread dough colored with magenta, aluminum powder, gentian violet, or methylene blue was tried; but the fluids of the digestive tract seemed to take the stain and carry it forward faster than the solids passed, thus staining everything on the way and leaving the original material behind. It was found, however, that fluids pass more rapidly through the canal than solids and that the rate of the passage varies directly with the quantity of fluid consumed. White and black oats were finally used and were found to be easily distinguishable in the feces. According to Browne's report, oats fed when the crop is empty appear in the feces within 5 to 6 hours, the time varying usually not more than an hour. The crop filled with oats empties within 18 to 20 hours. An entire meal of oats is passed within 27 to 28 hours. In two experiments in which birds were killed 20 and 10 minutes after they had begun to eat oats, a few oat grains were found in the gizzard. Retention of feed in the crop varies with the amount consumed, the more fluid portions passing out first. Liquids do not pass entirely through at once, although some portion passes immediately into the intestine. Liquids are not retained in the gizzard.

Kaupp and Ivey (*4, p. 10; 5*) used practically the method discarded by Browne. A mash colored by lampblack or a dye was fed, and the time at which the stain was last seen, together with that at which it first appeared in the droppings, was noted. Neither methylene blue nor gentian violet was found to be satisfactory, because these dyes seemed to cause constipation, which was followed by diarrhea. Lampblack gave more satisfactory results. From the averages of their results on two to six birds of each kind, the writers summarize their observations as follows:

Digestive processes of the fowl are rapid. The greatest rapidity is shown in the laying and in the growing fowl, food passing on an average of 3 hours and 52 minutes in the case of growing fowls and 3 hours and 46 minutes in the cases of the laying hens. Next in activity comes the adult hen not in laying condition, averaging 8 hours, and then the broody hen required an average of 11 hours and 44 minutes.

The valuable bulletin of E. W. Brown on the subject of digestion in poultry (*1*) gives some data on the retention of food in the crop, which may be summarized as follows: 30 gm. of corn fed in the morning into the empty crops of each of two fowls weighing 3 pounds, 5 ounces had entirely passed into the gizzard within 11 hours and 30 minutes and 12 hours and 15 minutes; the same quantity of corn fed the following morning had passed into the gizzard within 13 hours, and 13 hours and 30 minutes; 35 gm. fed at night had entirely disappeared within 15 hours, and 15 hours and 30 minutes; 50 gm. fed at 2 p. m. disappeared in 24 hours, 15 minutes and 24 hours, 25 minutes. These observations are said to be typical of a large number made with several fowls. The period of retention of oats in the crop was essentially similar. The figures indicate that larger amounts of feed require a longer time to leave the crop and individual birds of equal weight show slight variations.

Schwarz and Teller (*7*) tested the retention of feed in the crop, using eight hens and feeding to them four different grains, namely, wheat, barley, maize, and oats. Before the experiments began the hens were allowed to fast for 24 hours. Thirty grams of the grain was then fed to them and no other feed was given during the experiment, though water was always at hand. The presence of grain in the crop was determined by palpation. At first the palpation test was tried once an hour, but after the fifth time once a half-hour. The time that the feed remained in the crop varied greatly between different individuals and even between different tests on the same bird; but in general when 30 gm. of grain was fed the crop emptied itself in about 11 to 12 hours. The average length of time for the four grains was: Wheat, 11 hours; barley, 10 hours, 50 minutes; maize, 11 hours, 50 minutes; and oats, 12 hours, 20 minutes. The actual variation observed was from 5 to 15 hours. So wide a variation in the individual figures detracts from the significance of the averages. While grain is in the crop it absorbs moisture and swells. The effect of this absorption was studied by removing wheat grains from the crop at the end of 4, 6, and 8 hours and comparing their weight with that of similar grains which had not been ingested. The gain in weight of wheat staying in the crop for 4 hours was 11.43 per cent; of that staying 6 hours, 32.69 per cent; and of that staying 8 hours, 36.71 per cent.

Quite recently Steinmetzer (*8*) has reported observations with Röntgen-ray apparatus. Uniform pellets were prepared from a

definite weight of the feed (groats) mixed with an equal weight of barium sulphate and a little water, the barium sulphate allowing the position of the feed at any time to be seen and to be photographed. The hens fasted for 24 hours before the experiments. At the time of the introduction of the pellets and immediately following, observations were made under the rays at first once in 5 minutes, then once in a quarter of an hour, and finally once an hour. Both the crop and the gizzard were visible when empty and their movements in response to the feeds could be watched. As the pills were dropped in, some of them slipped directly past the crop into the gizzard, though the majority remained in the crop for a time. All stopped for about 0.5 to 1 minute at about 1 to 2 finger-widths from the shadow of the gizzard and then slipped into the gizzard. This pause evidently indicated a short stop in the glandular stomach. In general, the passage of the feed from part to part was, in this case, quite rapid, as is shown by the following average figures of the intervals after feeding at which the food appeared in and disappeared from the segments.

Segment	Interval after feeding	
	First appearance of the food	Final disappearance of the food
Crop	0	2 hours.
Gizzard	0.5–1.0 minute	2 hours 15 minutes.
Small intestine	15 minutes	3 hours.
Large intestine	2 hours	4 hours

The author thus summarizes his conclusions (*8, p. 505*):

The passage of the food through the gastrointestinal canal of the cock is normally exceedingly rapid; in the case of the food used in our experiments in pill form, in 4 hours. The feed remains in the crop, the gizzard, and the large intestine for equal intervals of time (in our experiments an average of 2 hours), in the small intestine about half as much longer (in our experiments an average of 3 hours). The gizzard is without movement when it is in an empty condition, but begins rhythmic movements as soon as food passes to it. The glandular stomach was always found to be empty except for the short moments when from time to time portions of the food passed through it (0.5 to 1 minute). The behavior of the crop was peculiar. Although the attempt was made in all the experiments, no rule could be discovered as to when the pill would fall into the crop and when not.

Data obtained with these small amounts of treated feed in pill form are instructive, though they can not be applied directly to the conditions of practical feeding. One experimental series with twice the amount of feed gave about twice as long intervals.

In connection with a careful study of the movement and digestion of starch broth in the different parts of the digestive tract of pigeons, Mangold (*6*) noted that in the cloaca and throughout the length of the intestine, indigestible hulls of grain were always to be found, together with small stones from the gizzard, even after the pigeons had been fed only starch broth for 3 to 6 days. This fact indicates that such indigestible pieces may remain for a long time in the gizzard even in large amounts, to be passed later into the intestine.

In the experiments to be reported here, a study was made of the dry weight and the moisture content of the material found in the different segments of the digestive tract of hens at various intervals

after the ingestion of three types of feed in common use, namely, whole corn, ground corn, and ground corn combined with a protein concentrate.

PLAN OF THE EXPERIMENTS

After a fast of 60 hours, during which time water was accessible but not food or grit, hens were individually fed 50 gm. of the feed to be observed and were then kept in separate cages, without access to food, for different intervals of time. Any feed remaining uneaten after 30 minutes was force fed. In the first experiment water was kept before the birds after the feeding. In the second experiment 75 c. c. of water was given to each bird in connection with the feeding, 50 c. c. being mixed with the ground feeds and the rest being introduced into the crop by means of a pipette. For the whole-corn group, 75 c. c. of water was introduced by pipette after the feeding of the corn. At the end of the desired time the birds were killed, the digestive tracts immediately removed—care being taken not to disturb the contents—and the contents of the several segments placed in weighed evaporating dishes and weighed as promptly as possible. The sections selected were: (1) Crop and tubes down to the proventriculus, (2) gizzard and proventriculus, (3) small intestine from gizzard to caeca, (4) caeca, and (5) large intestine including cloaca. In the first experiment (4) and (5) were combined. The excreta were also collected, dried, and weighed.

The contents of each segment were dried at 105° C. and the moisture determined. To insure greater accuracy in finding the weight of the feed in the gizzard, the whole material from the gizzard was incinerated in the muffle and the stones were separated as completely as possible from the ash and their weight was taken as the weight of the grit. The dry weight of the contents minus the weight of the grit is given as the dry weight of feed in the gizzard.

RATE OF PASSAGE OF FOOD AS INDICATED BY THE WEIGHT OF DRY MATTER IN SEGMENTS OF THE DIGESTIVE TRACT AT VARIOUS INTERVALS AFTER FEEDING

EXPERIMENT 1: WHOLE CORN

In the first experiment 50 gm. of whole corn was fed to each hen and the birds were killed at intervals of 1, 2, 4, 7, 12, 15, 24, and 48 hours afterward and examined. For comparison, another hen was killed and examined in the same way after a fast of 60 hours following the usual green feeds. The weights of dry matter found are given in Table 1.

The data indicate that the corn left the crop rather rapidly at first and then more slowly while gizzard digestion was going on, more than 12 hours being required for it to pass entirely from the crop. In the hen which was killed at the end of one hour three corn kernels were found which had not yet reached the crop. From the data in Table 1 it seemed that by the end of 15 hours the amount of dry material in the crop, gizzard, and large intestine had returned approximately to a fasting level. The amount in the small intestine showed no regular change. The average amount in the small intestine was 3.87 gm. and in the large intestine, 1.36 gm.

Table 1.—*Weight of dry matter in sections of the digestive tract and in the excreta of hens fed whole corn; experiment 1*

Hours after feeding	Weight in grams of contents of—						Remarks
	Crop	Gizzard		Small intestine	Caeca and large intestine	Weight of feces (grams)	
		Dry matter	Dry matter not grit				
1	37 66	16. 84	8. 54	3. 50	1 26	0	Much whole corn in tubes, as well as crop; 6 whole kernels in gizzard.
2	34 88	22.31	9. 18	4 84	1 49	0	1 whole kernel in gizzard.
4	29. 21	16.41	6. 14	4.14	2. 13	2. 81	Much water in crop, 1 whole kernel in gizzard.
7	19. 25	24. 21	12 81	4. 49	2. 50	4. 07	4 whole kernels in gizzard.
12	22 21	31. 77	10. 38	2. 92	1. 26	4 54	25 whole kernels in gizzard.
15	1.87	16. 60	6 72	4.97	1.36	6. 88	Crop showed only secretion; no whole kernels in gizzard.
24	. 04	15. 93	5 14	2. 88	. 84	11. 99	No appearance of corn anywhere
48	. 03	12. 79	5. 84	2. 52	1. 15	18. 24	No appearance of corn.
60 (control)	45	18. 25	9. 94	4. 56	29		Straw in crop, grass in gizzard.
Average				3. 87	1. 36		

EXPERIMENT 2: WHOLE CORN, GROUND CORN, AND GROUND CORN PLUS TANKAGE

In Experiment 2 the hens were fed whole corn, ground corn, and a mixture of ground corn and tankage in the proportion of 4 to 1. The experiment was carried out as before with the exception of the intervals allowed after feeding and before killing the birds. In order to obtain more complete information on the later periods of digestion without multiplication of birds, it was decided to obtain data for the time at which the last of the feed left the crop and at intervals of 1, 2, 3, and 4 hours thereafter. The time at which the crop emptied was judged by external manipulation, and the hens were killed accordingly. This method of estimating the condition of the crop did not prove satisfactory for the kind of study desired. Hence the data will be considered entirely with regard to the interval after feeding and without regard to the time after the crop appeared to be empty. The weights of dry matter obtained in experiment 2, arranged on that basis, are given in Table 2.

In a consideration of this larger collection of data, the great irregularity in the results is at first most striking. Wide variations among the individual birds occur which can not be attributed to the difference in the intervals which elapsed after feeding. For instance, one bird of the whole-corn group had 34 kernels (small ones) in the crop after 23 hours, and one of the ground-corn group had 5 gm. of dry matter in the crop at the end of 35 hours. This last amount is nearly the same as was found in a hen of the same group after 12 hours. One factor which partly explains the irregularities and at the same time complicates the interpretation of the data is the uncertainty as to the amounts of the secretions present. When a crop contains nothing but its own secretions it may show nearly 2 gm. of dry matter. We have no means of judging the quantity of secretion present in the other sections of the digestive tract. After the longer periods of digestion the secretions probably make up larger proportions of both the dry matter and the moisture present.

TABLE 2.—*Weight of dry matter in sections of the digestive tract and in the excreta of hens fed whole corn, ground corn, and ground corn and tankage; experiment 2*

WHOLE CORN

Hours after feeding	Weight in grams of contents of—						Weight of feces (grams)	Remarks
	Crop	Gizzard		Small intes-tine	Caeca	Large intes-tine		
		Dry matter	Dry matter not grit					
11.3	0.56	8.42	7.85	4.04		a b 0.65	3.05	Crop 2 whole kernels. / Gizzard 15 whole kernels.
12 8	.45	11.41	6.10	3 91	1.21	.43	6 00	Crop 1 whole kernel. / Gizzard 3 whole kernels.
15.4		7.02	b .24	1.64	.63	.39	5.27	Crop 1 whole kernel. / Gizzard no whole kernels.
19.7	.91	9.74	6.84	2 57	.63	.89	4.22	Crop no whole kernels. / Gizzard no whole kernels.
20. 2	.76	19.02	8 19	2.09	.18	.26	6.01	Crop 1 whole kernel. / Gizzard 5 whole kernels.
21 1		10.63	4.94	2 05	(e)	.23	4.94	Crop no whole kernels. / Gizzard 2 whole kernels.
21.1		13 22	3.39	1.37	.78	.20	4 28	Crop no whole kernels / Gizzard no whole kernels.
23.3	7.08	16.54	4.87	2.40	.73	.19	3.77	Crop 34 whole kernels / Gizzard 2 whole kernels
Average			6.03	2.51	.69	.37		

GROUND CORN

11.8	4.33	8.98	6.23	5.68	0.59	0.57	3 06	
13.5	3.73	20 60	5.46	3 53	1.66	1.03	7.87	
15.0	2.72	10.28	4.84	2 17	.66	.25	4.05	
16 1	14.14	12.64	4.78	3.34	.78	.89	1.54	
18 5	.63	11.88	5.33	2 52	.38	.14	5.38	
34 8	4 97	16 82	7.91	3 35	.71	.90	7.04	
Average			5.76	3.33	.80	.63		

GROUND CORN AND TANKAGE

11.6	2 71	13 72	6.64	5 53	0.34	0 38	5.87	
13.2	2.58	17.72	5.53	4 23	.33	(e)	5.47	
19.4	.39	15.48	7.36	3.86	.77	74	.99	
20.5	1 25	10.12	4.86	2.26	.68	.48	7.12	
23.2	.23	20.20	5.36	1.97	51	(e)	4.90	
24 2	.86	9.82	6.17	2.24	34	.67	6.24	
25 1	.37	15 40	5.90	4.45	1.00	1.22	3.90	
26.1	.90	10 66	3 58	3.84	.39	(e)	4 71	
Average			5.68	3.55	.55	.70		

a Large intestine + caeca.   b Not included in the average   e Lost.

Some interesting and significant facts are brought out if we consider not individual birds but the conditions found during periods of several hours each. In Tables 3 and 4 the data of experiments 1 and 2, respectively, have been arranged to permit of such a study. The periods selected are those covered by the hours 1 to 4 (experiment 1), 5 to 10 (experiment 1), 11 to 14 (experiments 1 and 2), 15 to 21 (experiments 1 and 2), 22 to 28 (experiments 1 and 2), and more than 30 (experiments 1 and 2).

Table 3.—*Average weight of dry matter in sections of the digestive tract of the hens during successive periods after feeding; feed, whole corn; experiment 1*

	Weight in grams of contents of—			
Hours after feeding	Crop	Gizzard	Small intestine	Caeca and large intestine
1–4 [a]	33.9	8.0	4.2	1.6
7	19.3	12.8	4.5	2.5
12	22.2	10.4	2.9	1 3
15	1.9	6 7	5.0	1.4
24	.04	5.1	2.9	.8
48	.03	5.8	2.5	1.1
60 (control)	.5	9.9	4.6	.3

[a] 3 birds; in each of the other cases, 1 bird.

Table 4.—*Average weight of dry matter in section of the digestive tract of hens during successive periods after feeding; feed, whole corn, ground corn, and ground corn and tankage; experiment 2*

Hours after feeding	Crop			Gizzard			Small intestine			Caeca			Large intestine		
	Whole corn	Ground corn	Corn and tankage	Whole corn	Ground corn	Corn and tankage	Whole corn	Ground corn	Corn and tankage	Whole corn	Ground corn	Corn and tankage	Whole corn	Ground corn	Corn and tankage
11–14	[a]0 5	[a]4 0	[a]2.6	[a]7.0	[a]5.8	[a]6.1	[a]4 0	[a]4.3	[a]4.9	[b]1 2	[a]1.1	[a]0.3	[b]0.4	[a]0 8	[b]0.4
15–21	[a].8	[e]5.8	[a].8	[d]4 7	[e]5.0	[a]6 1	[d]1.9	[c]2.7	[a]3.0	[e].6	[e].6	[a].7	[d].4	[e].6	[a].4
22–28	[b]7 1		[e].6	[b]4.9		[e]5.3	[b]2 4		[a]3.1	[b].7		[e].6	[b].2		[a].9
35		[b]5 0				[b]7.9		[b]3.4			[b].7			[b].9	

[a] 2 birds.   [b] 1 bird.   [c] 3 birds.   [d] 5 birds.   [e] 4 birds.

From a consideration of these tables, representing the two experiments, the following conclusions seem to be justified:

Crop. All whole corn leaves the crop by the end of 12 to 15 hours. Ground corn leaves the crop more gradually, with the result that when ground corn is fed a much longer time is required for the crop to become empty. Ground corn and tankage mixed in the ratio of 4 to 1 leave the crop more slowly than whole corn but more rapidly than ground corn alone.

Gizzard. The amount of dry matter in the gizzard in addition to the grit is larger when there is feed in the crop than when there is none, but otherwise it does not vary greatly. Nor does it vary with the three types of feed used.

Small intestine. The amount of dry matter in the small intestine is the same for the three feeds, and is somewhat higher during the first 15 hours after feeding than it is later.

Large intestine and caeca. The amount of dry matter in the large intestine and the caeca probably does not change very much during digestion, though it may rise somewhat 5 to 10 hours after feeding (See Table 1.) The figure for the control hen of experiment indicates that it may fall markedly during a prolonged fast.

MOISTURE IN THE DIGESTIVE TRACT OF HENS

The data collected reveal some interesting facts with regard to the degree of moisture present in the different sections of the digestive tract of the hen. Table 5 shows the percentages of moisture found in experiment 1 and Table 6 those found in experiment 2.

TABLE 5.—*Percentage of moisture in the contents of sections of the digestive tract of hens fed whole corn; experiment 1*

Hours after feeding	Percentage of moisture in the contents of—			
	Crop	Gizzard	Small intestine	Caeca and large intestine
1	23. 2	43. 9	84. 7	77. 7
2	17. 9	35. 9	86. 4	83. 1
4	49 9	36. 8	85. 0	82. 4
7	38. 7	47. 8	83. 2	79. 6
12	20. 6	32. 4	85. 1	80. 9
15	52. 8	46. 1	82. 0	81. 9
24	95 1	34. 5	82. 5	92. 9
48	95. 0	44. 7	83. 9	86. 7
60 (control)	74. 5	47. 7	76. 8	76. 1
Average		41. 1	83. 3	82. 4

TABLE 6.—*Percentage of moisture in the contents of sections of the digestive tract of hens fed whole corn, ground corn, and ground corn and tankage; experiment 2*

WHOLE CORN

Hours after feeding	Percentage of moisture in the contents of—				
	Crop	Gizzard	Small intestine	Caeca	Large intestine
11.3	69. 7	57. 3	82. 6	[a] 80. 2	
12.8	67. 6	47. 6	82. 2	69. 0	79. 2
15.4		54. 6	75. 0	80. 3	86. 2
19 7	80. 1	58. 2	84. 4	79. 5	77. 7
20.2	88. 6	45. 4	85. 1	77. 2	82. 6
21.1		43. 4	86. 8	([b])	80. 1
21.1		34. 9	82. 6	71. 1	81. 5
23 3	54. 4	40. 8	84. 0	70. 9	72. 0
Average		47. 8	82. 8	74. 7	79. 9

GROUND CORN

Hours after feeding	Percentage of moisture in the contents of—				
	Crop	Gizzard	Small intestine	Caeca	Large intestine
11.8	61. 4	56 6	82. 2	69. 7	77. 0
13.5	60. 2	31. 1	83. 9	73. 2	67 6
15.0	62. 3	50. 4	82. 4	78. 5	78. 6
16.1	53. 5	48. 4	80. 5	69. 5	74. 6
18 5	78. 5	39. 5	83. 3	79. 9	75. 9
34.8	61. 5	46. 5	85. 8	80. 5	77. 4
Average		45. 4	83. 0	75. 2	75. 2

GROUND CORN AND TANKAGE

Hours after feeding	Percentage of moisture in the contents of—				
	Crop	Gizzard	Small intestine	Caeca	Large intestine
11.6	67. 2	47. 0	80. 9	78. 1	78. 7
13.2	74. 2	34. 4	83 2	76. 8	([b])
19.4	68. 9	43. 4	86. 8	80. 8	85. 0
20.5	65. 6	46. 4	84. 5	71. 7	81. 2
23.2	91. 3	30. 9	80. 1	80. 9	([b])
24.2	74. 6	54 9	82. 6	79. 8	82. 1
25.1	77. 7	45. 3	81. 6	77. 5	82. 6
26.1	74. 7	41. 7	81. 8	82. 4	([b])
Average		43. 0	82. 7	78. 5	81. 9

[a] Caeca+large intestine; not included in the average.   [b] Lost.

TABLE 7.—*Amount of water in the crop and gizzard, and ratio of moisture to dry matter not grit in gizzard*

EXPERIMENT 1· WHOLE CORN

Hours after feeding	Water in—		Ratio of moisture to dry matter not grit in gizzard
	Crop (grams)	Gizzard (grams)	
60 (fast)	1.3	16.7	1 7 · 1
1	11 3	13.2	1 6 1
2	7.6	12.5	1 4 · 1
4	ª29 1	9.5	1 6 . 1
7	12.1	22.2	1 7 · 1
12	5.8	15.2	1.5 1
15	2.1	14.2	2 1 · 1
24	.8	8.4	1.6 : 1
48	.6	10.3	1.8 : 1

EXPERIMENT 2: WHOLE CORN

11	1.3	11.3	1 4 : 1
13	.9	10.4	1 7 : 1
15	ª2.2	8.4	ª 35 1 : 1
20	3 7	13.6	2.0 . 1
20	5 9	15.8	1 9 . 1
21		7.7	1 6 · 1
21		7.1	2 1 : 1
23	8. 5	11.4	2 3 : 1

EXPERIMENT 2· GROUND CORN

12	6. 9	11.7	1.9 . 1
14	5.7	9.3	1 7 : 1
15	4.5	10.5	2.2 : 1
16	16 3	11.9	2 5 : 1
19	2.3	7.8	1.5 . 1
35	8.0	14.6	1.8 : 1

EXPERIMENT 2: GROUND CORN AND TANKAGE

12	5. 6	12.2	1 8 . 1
13	7.4	9.3	1 7 : 1
19	.9	11.9	1.6 : 1
21	2.4	8.8	1.8 . 1
23	2 4	9.0	1 7 : 1
24	2.5	12.0	1.9 : 1
25	1.3	12.7	2 2 : 1
26	2.7	7.6	2.1 : 1

ª Much visible water.   ᵇ Very little food residue.

The quantity of moisture in the crop is very variable, and in these experiments shows no relation to the type of feed eaten or to the interval after feeding. In experiment 1, where water was kept before the birds all of the time, the amount in the crop may have been affected by the time after drinking. In some cases water was distinctly evident in the crop, either as water drunk or as secretion. In the other sections, aside from the gizzard, the percentage of moisture remained nearly constant. In the gizzard the moisture content was rather variable, though less so than in the crop. An inspection of Tables 5 and 6 shows strikingly that the percentage of moisture in the gizzard is not a reflection of that in the crop. For example, two birds in experiment 1 each showed 48 per cent moisture in the gizzard, but the amount in the crops of the same birds varied as widely as from 75 to 39 per cent; one bird of the corresponding group in

experiment 2 which had 45 per cent moisture in the gizzard, showed 89 per cent in the crop. The same lack of uniformity in moisture content of crop and gizzard is apparent if one considers the actual amount of moisture in these organs instead of the percentages. The amount of moisture in the crop seems to be absolutely independent of its other contents and that in the gizzard is usually about 1.5 to 2 times the amount of dry matter not grit present (Table 7).

The percentages of moisture present in the several sections of the digestive tract may be summarized thus: (1) In the crop an indefinitely variable amount; (2) in the gizzard about 30 to 60 per cent (the general average for the four groups was 44.2 per cent); (3) in the small intestine about 82 to 86 per cent (the average in each group was 83 per cent); (4) in the caeca 70 to 80 per cent (the general average for three groups was 76.4 per cent); and (5) in the large intestine 75 to 85 per cent (the general average for three groups was 78.9 per cent). The average percentage in the caeca and large intestine of the hens in experiment 1 was 82.4 per cent. The percentage was remarkably constant in the small intestine. As was to to be expected, the small intestine showed the highest percentage generally and the large intestine next. The moisture content of the caeca was slightly lower than that of the large intestine. The moisture content of the gizzard was distinctly low.

GRIT IN THE GIZZARD OF HENS

E. W. Brown (*1*) undertook a study of the utilization of sand by poultry. Comparisons were made of the SiO_2 consumption and excretion, and in some cases the gravel found in the crop and gizzard was weighed and examined. With free access to gravel the amount of both the ingestion and excretion varied widely with the individual birds and with the type of feed. The values were considerably higher on an oat diet than on a corn diet. Under such conditions the excretion sometimes reached 6 gm. of sand per day; but when no grit was available, the excretion fell to a low figure.

T. G. Browne (*2*), performing experiments in which sand and shot were mixed with the food when the normal amount of grit was otherwise provided, found that this excess sand and shot appeared in the feces in the 5-hour interval usual for food. On the other hand, he found the gizzard rarely if ever free from sand and pebbles even after the birds had been without access to grit for six weeks.

A few observations on the amount of grit in the gizzard were made by Buckner and Martin (*3*) in connection with a study of the formation of bone and egg shell from different mineral sources. After eight months the lot receiving nothing but grain and tankage showed 12.6 gm. of grit in the gizzard.

Kaupp and Ivey (*4, 5*) made similar observations, reporting grit present in the gizzard even after a year without opportunity for renewal, one hen showing 5.89 gm. of grit after 365 days. The birds still appeared healthy, indicating that the retained grit was sufficient to grind the feed.

All of these observers have found grit constantly present in the gizzard, but in very different quantities. Their work also gives evidence that grit may normally be found in all sections of the digestive tract and in the excreta. It appears, however, that when there is no opportunity for the chicken to pick up grit, whatever is in the gizzard is retained there for a long time.

In the experiments reported here no attempt was made to estimate the amount of grit or sand in any portion of the tract except the gizzard. The probable presence of sand is, however, one of the factors contributing to the complexity of the problem and may explain in part the irregularities of the results obtained. In the gizzard the grit makes up so large and so variable a part of the total dry matter that a rough determination of the amount was made by ashing at low red heat the total contents of the gizzard, sifting out with a 40-mesh sieve the fine powdery ash, and weighing the residue as grit. The weights so obtained are given in Table 8. The birds had all been without access to grit for 60 hours previous to the beginning of the experiment.

TABLE 8.—*Grit content of the gizzard of hens used in experiment 1 and experiment 2*

	Experiment 1		Experiment 2					
	Whole corn		Whole corn		Ground coin		Corn and tankage	
Hours after feeding	Grit (grams)	Hours after feeding	Grit (grams)	Hours after feeding	Grit (grams)	Hours after feeding	Grit (grams)
60 (fast)	8.31	11	0.57	12	2.75	12	7.08
1	8.30	13	5.31	14	15.14	13	12.19
2	13.13	15	6.78	15	5.44	19	8.12
4	10.27	20	2.90	16	7.86	21	5.26
7	11.40	20	10.83	19	6.55	23	14.84
12	21.38	21	5.09	35	8.91	24	3.65
15	9.88	21	9.83			25	9.50
24	10.80	23	11.67			26	7.08
48	6.95						
Average	11.16		6.62		7.77		8.47

Table 8 shows a rather wide variation in the amount of grit in the gizzards of the hens. In one case there was as little as 0.57 gm. and in another case as much as 21 gm.; but in general the amount ranged from 3 to 15 gm. No connection was observed between the amount of grit present and any other factor or condition.

SUMMARY

Determinations have been made of the amount of dry matter contained in the various segments of the digestive tract of hens at different intervals after the ingestion of 50 gm. of whole corn, ground corn, or a mixture of ground corn and tankage in the ratio of 4 to 1.

The results indicate a wide variability in the rate of passage of the food through the hen. In most cases, however, the whole corn had left the crop by the end of 12 to 15 hours. The ground corn remained in the crop distinctly longer than the whole corn and somewhat longer than the mixture of corn and tankage. The amount of dry matter in the other sections of the digestive tract did not differ notably for the three kinds of feed used. The amount of dry matter in the gizzard in addition to the grit was larger while food remained in the crop than later, and to a less extent the same was true of the small intestine. Otherwise the amount of dry matter in the intestines was fairly constant.

The percentage of moisture in the contents of the crop showed a wide variation, apparently independent of the kind of feed eaten and of the time after feeding. The percentage of moisture within the gizzard was low and rather variable, being about 30 to 60 per cent, with an average of 44.2 per cent. The amount was not a reflection of the amount in the crop, but was usually about one and one-half to two times that of the dry matter not grit present. The percentage of moisture within the small intestine was high and remarkably constant at about 82 to 86 per cent, with an average of 83 per cent. That in the caeca was about 70 to 80 per cent of the total contents, with an average of 76.4 per cent; and that in the large intestine was about 75 to 85 per cent, with an average of 78.9 per cent.

The amount of grit found in the gizzard varied widely, the usual range being from 3 to 15 gm. No connection was observed between the quantity of grit present and any other factor or condition.

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[TH]E FACTORS OTHER THAN BACTERIA THAT INFLU-ENCE THE BODY OF ARTIFICIAL BUTTERMILK [1]

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INTRODUCTION

"The practice of adding sodium citrate to milk used as infant food," according to Van Slyke and Bosworth (3)[3] "has been common for many years. It has found application especially in the treatment of certain types of 'feeding-cases' in which untreated milk, after entering the stomach, forms abnormally large chunks of tough curd, shown by Talbot to consist of casein * * *. Empirical practice has shown that this abnormal curdling of milk may, to some extent, be modified or controlled by the addition of sodium citrate at the rate of 1 to 2 grains per ounce of milk."

To explain the mechanism of this practice on a chemical basis, those investigators conducted some experiments in which they showed that when sodium citrate is added to normal milk the time of coagulation is increased. The curd increases in softness with an increase in the amount of added citrate, and when as much as 0.4 gm. per 100 c. c. of milk is added, coagulation does not take place. They have also shown that there is an increase in the quantities of soluble calcium, magnesium, and phosphorus, and that the increase in soluble calcium is mainly at the expense of the casein molecule.

Later, Bosworth (1, 2) published several papers dealing with the question of milk for infant feeding. He studied human milk and determined its probable chemical composition in the same manner that cow's milk was formerly studied by Van Slyke and himself. He reports that when rennet is added to it human milk will give a small amount of finely divided precipitate. The difference in the behavior of human milk from that of cow's milk is explainable, according to Bosworth, by the difference in the base content of both milks. He found a 7.2 gm. equivalent of calcium for each 10 gm. equivalent of sod iumand potassium in human milk, which is in contrast to a 13.2 gm. equivalent of calcium for each 10 gm. equivalent of sodium and potassium, found in cow's milk. Bosworth is inclined to believe that the casein of human milk is in the form of calcium potassium caseinate. In other words, the addition of sodium salts to cow's milk results in the prevalence of sodium and potassium over calcium and magnesium, thus changing the calcium caseinate of cow's milk to calcium sodium caseinate or simply to sodium caseinate, which resembles the casein of human milk in its characters.

Bosworth's investigations and conclusions suggested to the the possibility of improving the body of commercial buttermilk in this country from cow's skimmed milk by the addition to it of a certain quantity of a sodium salt. The manufacturers of this product have always experienced difficulty in preventing wheying off, and attempts to obtain a sure and practical method of manufacturing which would eliminate daily variations in the quality of the product have not been successful. Present methods of manufacturing vary with the plants and are largely empirical. The purpose of the investigation herein discussed was to study the possibility of developing, on a scientific basis, a method of manufacturing buttermilk which would eliminate the daily variations in the quality of that product. Such a venture is important from an economic as well as from a scientific point of view.

PROCEDURE AND TECHNIC

Given two different samples of buttermilk, how can one determine the quality of each more reliably and accurately than by the ordinary judging method? How can the inherent qualities of each sample be transformed into tangible elements, comparable even within narrow limits? That was the primary problem to be solved in this study. The value of the conclusions depends to a large extent on the accuracy with which the quality of each sample has been determined.

It was recognized in the beginning that there are in general three main characters which largely determine the quality of buttermilk. These are: (1) The texture of the product, i. e. the size and arrangement of the particles which make up its body; (2) the consistency of the product, i. e. the manner in which its particles adhere or are cemented together; and (3) the flavor of the product.

In this study the amount of the whey liberated was estimated in all instances and was taken into consideration before any conclusion was drawn.

The texture of the product was determined by the weight of particles above a certain arbitrary size. The separation of the particles into two classes was done by sedimentation. Three hundred cubic centimeters of water were measured into a 500 c. c. Erlenmeyer flask, into which 100 c. c. of the broken coagulum was poured slowly and carefully, as well as 50 c. c. of water, used to rinse the measuring cylinder. The object of adding water was to destroy the viscosity of the buttermilk as far as possible so that the lumps might settle freely. The flask was then rotated very carefully 10 times in order to mix its contents, and allowed to stand for one minute, so that the lumps could settle in the order of their coarseness. At the end of one minute the supernatant, and the the settling lumps, were poured over a Buchner funnel. In addition the flask was rinsed with 50 c. c. of water and the resulting contents poured into the funnel. This process separated the particles into two groups, the fine particles which passed through the openings, and the lumps which were retained on the funnel, from which they were washed with a gentle stream of water into a previously weighed, dry square of cheesecloth. The cheesecloth and its contents were allowed to dry for 24 hours in the air, when the weight of the dry lumps was determined.

The procedure is simple. It is difficult, however, to obtain accurate results with it, owing to the perishability of the lumps. The

st uniformity is necessary, therefore, in breaking up the
lum and in the subsequent handling such as pouring and
rotating. When carefully performed, the procedure may be said to
b accurate to within 10 per cent.

The consistency of the product obtained from this process was
measured by recording the time it took a plunger to travel a pre-
scribed distance downward through a column of the product, on
the theory that the consistency of a body is proportional to the
resistance that body offers to a shearing force applied to it. A glass
cylinder (C) was filled with the sample to a certain mark, and the
plunger was placed so that its upper side was just below the surface,
and allowed to sink. The more viscous the product, the longer
it took the plunger to travel the same distance. The best disposi-
tion was found to be the one represented in
Figure 1. The shearing force π is equal to
the weight p of the plunger P, minus the
sum $p' + p''$ where p' is the weight of the
counterweight P', and p'' the weight of a
volume of the product equal to the volume
of the plunger.

$$\pi = p - (p' + p'')$$

The difference $p - (p' + p'')$ may be varied
at will so that the sinking velocity of P may
be reduced to the best advantage. In the
apparatus used π was such that it took P
10 seconds to travel the same distance (l)
in an 18 per cent gelatin solution of a specific
gravity equal to 1.038 at 30° C.

While this method of determining vis-
cosity may give very accurate results with
liquids of homogeneous character, its accuracy
in testing the viscosity of buttermilk has been
found to depend to a great extent on the tex-
ture of the sample of buttermilk. The larger

Fig. 1.—Outline of apparatus used
to determine the consistency of
buttermilk: C, glass cylinder; P,
plunger; P', counterweight; l, dis-
tance traveled by the plunger

the lumps, the greater, usually, was the viscosity recorded. This is
to be expected, as those lumps include a large amount of water, so
that the volume V of particles per cubic centimeter in Einstein's
formula ($n_s = n_m$ (1 + 2.5 V)) will be increased. As a consequence
therefore, the figures representing viscosity may not add to the infor-
mation obtained from the weight of the lumps. Nevertheless, vis-
cosity determination has been found of great value in instances
where a relatively coarse body has a very poor consistency, as in the
case of buttermilk made from milk heated to 145° F. for 30 minutes,
at which temperature the body gets too weak to measure viscosity.
A viscosity of 20 or slightly above was found to correspond to the
optimum results.

The method usually followed in this study to test the effect of a
chemical in influencing changes in the body of artificial buttermilk
was to weight 0.1, 0.2, 0.3, 0.4, and 0.5 per cent portions of the chemi-
cal into 1-liter Erlenmeyer flasks to each of which 750 c. c. of raw
skimmed milk was also added. The flasks were plugged with cotton,

heated in an Arnold steam sterilizer to 100° C., and held at that temperature for 15 minutes. They were then cooled to 25° C. or below and inoculated with a pure culture of *Streptococcus lactis*,[1] after which they were usually incubated at room temperature which varied occasionally from 21° to 28°, but was most of the time in the neighborhood of 25°. At the end of 24 hours, during which the flasks were inspected occasionally, the coagula were broken by rotating 30 times, 15 clockwise and 15 anticlockwise, care being taken to observe as much uniformity as possible. One hundred cubic centimeters of the contents was then removed from each flask and the texture determined. The remaining portion served to determine the viscosity. A sixth flask to which no chemicals were added served as a control.

This general procedure was modified according to the object of the experiment.

In all instances the flasks were judged before and after the coagulum was broken and a record made of the quantity of free whey, and the character and flavor of the coagulum. The figures obtained by the methods above described were then compared. A stop watch was used whenever it was necessary to make time records.

PRELIMINARY EXPERIMENTS

Before the effects of the various salts were studied it was found necessary to determine how certain factors like the quantity of inoculum, the temperature to which the milk is heated, and the temperature at which it is incubated, would affect the quality of the product. If the effect due to a chemical is to be studied, those factors should be controlled, and the variations which they produce, once determined in degree and direction, should be eliminated. It was believed also that such variations might be utilized for the improvement of the product.

EFFECT OF QUANTITY OF INOCULUM ON QUALITY OF BUTTERMILK

It is not possible to state definitely what quantity of inoculum should be added to a given quantity of milk to get the best results. Theoretically, the quantity of inoculum should depend largely on the condition of the culture used for inoculation; the more virulent the culture, the smaller should be the quantity of inoculum added. In this work a highly virulent 24-hour-old culture was used most of the time, so that 0.05 c. c. of culture added to a liter of milk usually produced coagulation in from 12 to 15 hours. The results obtained are summarized in Table 1.

TABLE 1.—*Effect of quantity of inoculum on quality of buttermilk*

Flask No.	Inoculum per 750 c. c. of milk (c. c.)	Quantity of undesirable lumps (mgm. per 1,000 c. c.)		Viscosity in seconds		Percentage acidity as lactic acid	
		At 24° C.	At 28° C.	At 24° C.	At 28° C.	At 24° C.	At 28° C.
1	0.03	7	493	20.0	23.8	0.685	0.685
2	.1	8	694	24.1	28.6	.710	.690
3	.2	17	787	24.4			
4	.3		840	24.8		.720	.710
5	.4	21	1,302	25.2	28.1		
6	.5	29	1,485		29.4	.73	.720

Table 1 shows that, other things being equal, an increase in the quantity of inoculum causes an increase in the lump content and the titratable acidity of the product. Similarly an increase in the quantity of free whey and the firmness of the coagulum was observed to accompany an increase in the quantity of inoculum. The relative increase in these various factors at both temperatures used is in close agreement, but the magnitude of that increase becomes more and more important as the temperature of incubation is increased, so that for practical purposes the following conclusion may be drawn: At a low incubation temperature, the quantity of inoculum is a minor factor, but it increases in importance as the incubation temperature increases. A large dose of inoculum tends to render the product lumpy and more acid.

EFFECT OF THE DEGREE TO WHICH THE MILK IS HEATED, AND OF INCUBATION TEMPERATURE ON BUTTERMILK QUALITY

Seven hundred and fifty cubic centimeter portions of raw skim milk were measured into each of six 1-liter Erlenmeyer flasks. Two of the flasks were heated in an Arnold steam sterilizer at 100° C. for 15 minutes; two others were heated in a water bath at 82° for the same length of time; the remaining two flasks were heated in a water bath at 63° for 30 minutes. After cooling and inoculating, one of each series was incubated at 37° and the others left at room temperature. The flasks that were heated to 63° only did not coagulate within 24 hours and in most cases two or three days passed before any coagulum was formed. When such coagulum was broken, it had a sandy texture and an extremely weak consistency. The mixture was composed of a large number of isolated particles of a medium coarseness floating in the serum. The slow coagulation, together with a pronounced foreign flavor, suggested that the sandy texture and weak consistency were due to resistant bacteria which survived the Pasteurization temperature and hindered the multiplication of the lactic culture. Many organisms were accordingly isolated, some of which are spore formers. The flavor of the product and an examination of the colonies on a milk-powder agar medium showed that some of those organisms produce acid, while others are of the peptonizing type. Moreover, a certain colloidal factor also may have operated; the importance of the chemical and physical factors has been very much overlooked in the past.

There was a marked improvement in the body of the product in the remaining flasks when the milk was heated to 100° C. as compared to the product made from milk heated to 82°. The probable reason for that difference is discussed in another section of this article.

The tendency of the product incubated at 37° to whey off and to increase in lump content was especially noticeable. A bad flavor found in all of the flasks, and more pronounced in the flasks heated to 82°, was due, probably, to the action of some spore formers which survived and were able to multiply rapidly enough at 37° to cause a change in the flavor.

The tendency to whey off at a rather high temperature seems to be mainly a physical phenomenon. The structure of the coagulum, in all probability, is similar to that of a sponge; its mass is penetrated, in all directions, by a network of very fine capillaries which

hold the original serum of the milk. The capacity of those capillaries to hold serum decreases with rising temperature. That has been shown by Wolf (*6, p. 524*) who measured the capillary rise of various liquids up to their critical temperatures. The capillary constants decreased gradually and became nil near the critical temperature, while the meniscus disappeared and the surface of the liquid inside as well as outside of the capillary tube became horizontal. Because of the faster rate of acid production at that temperature, milk coagulates much faster at 37° C. than at room temperature. This rapid coagulation has a tendency to decrease the absorptive capacity of the coagulum, because capillary movement in very fine tubes is extremely slow due to the enormous friction inside such tubes. In order to make that point clearer the process of coagulation may be compared to that of crystallization. Both processes deal with particles of matter which come together; the main difference is in the magnitude of the particles. It is a well known fact that when crystallization takes place quickly the crystals formed contain only a small quantity of impurities, while a slow coagulation, on the other hand, results in the formation of impure crystals. The impurities, in the present case, consist of the serum and the salts dissolved in it. Throughout this study the beneficial effect of slow coagulation was observed. In all cases, when coagulation took place, it resulted in an improvement in the body.

Table 2 shows the effect of heat and incubation temperature on the quality of buttermilk, while Table 3 records the rate of acid production at ordinary and warmer temperatures.

An examination of Table 3 shows that acid production proceeds much faster at 37° C. than at room temperature, and that milk coagulates a few hours earlier at 37° than at room temperature. It reveals, moreover, the very interesting fact that, after coagulation takes place, the rate of acid production at 37° is reduced and that at room temperature it rises rapidly, so that the total amount of acid produced at room temperature finally exceeds that produced at 37°.

On the other hand, Loeb (*10*) has shown that the solubility of casein-acid salts is brought about by the forces resulting from Donnan's equilibrium. He observed that the solution of casein chloride particles in hydrochloric acid was preceded by an increase in swelling and he was able to measure that increase. He recorded, furthermore, a maximum solubility and also a maximum swelling at about P_H 2.0 of the outside solution. The higher acidity at room temperature therefore may be partly responsible for the softer curd and the fewer lumps observed at that temperature.

TABLE 2.—*Effect of heat and incubation temperatures on the quality of buttermilk*

Flask No.	Temperature at which heated	Temperature at which incubated (° C.)	Quantity of undesirable lumps (mgm.)		Viscosity in seconds	
			Series 1	Series 2	Series 1	Series 2
	° C.					
1	63	Room temperature	(a)	(a)	(a)	(a)
2	63	37°	(a)	(a)	(a)	(a)
3	82	Room temperature	1,532	1,231	32.2	34.4
4	82	37°	4,557	4,875		86.0
5	100	Room temperature	470	391	23.3	25.2
6	100	37°	4,486	4,012	54.6	57.2

a Did not curdle within 24 hours.

TABLE 3.—*Rate of acid production in milk at different temperatures of incubation*

Number of hours between inocula- tion and taking of acidity record	Percentage acidity in form of lactic acid		Condition of sample
	Room tempera- ture	37° C.	
9	0. 210	0. 250	Neither sample curdled.
12	. 495	. 675	Sample at 37° C. curdled.
16	. 725	. 770	Both samples curdled.
21	. 760	. 780	Do.
24	830	. 800	Do.

EFFECT OF SODIUM SALTS ON QUALITY OF BUTTERMILK

GENERAL EFFECT

The experiments to test the effect of sodium salts on the quality of buttermilk were performed in the manner described above with various sodium salts. The effect of the sodium as well as of the acid radicals was studied. The following general conclusions were drawn from these experiments:

1. All sodium salts studied, when added in the appropriate concentration, produced some improvement in the body of buttermilk.

2. The action of sodium salts is specific and represents the action of the sodium and of the acid radical.

3. When added in a certain concentration sodium salts alter the flavor. The extent of the change in flavor varies with the specific salt and the concentration in which it is added. Even tasteless salts altered the flavor of buttermilk. The concentration which produces the desired body has always been higher than that required to alter the flavor.

Titration experiments made as a part of this study, also point to the fact that at least some of the salts added to the milk increased the titratable acidity of the buttermilk. Table 4 illustrates some of the results obtained in the titration tests.

TABLE 4.—*Effect of sodium salts on the quality of artificial buttermilk*

Name of salt	Percent- age added	Quantity of unde- sirable lumps (mgm.)	Viscos- ity in seconds	Percent- age of acidity as lactic acid
Rochelle salt	0. 0	506	21. 5	
	. 1	296	22. 8	
	. 2	147	22. 0	
	. 3	89	21. 8	
	. 4	69	20. 4	
	. 5	76	20. 0	
Sodium chloride	0. 0	106	33. 6	0 623
	. 1	49	24. 6	. 635
	. 2	29		
	. 3	0	21. 2	. 650
	. 4	0	18. 4	
	. 5	0	17. 6	. 655
Sodium nitrate	0 0	459	34. 0	0. 623
	. 1	337	31. 2	
	. 2	291	28. 4	
	. 3	183	24. 6	. 685
	. 4	107	22. 3	
	. 5	92	21. 4	. 706

Other sodium or potassium salts used in the test include the malates, the carbonates, the phosphates, the acetates, and the mucates. It was not deemed necessary to report the detail of the figures obtained, as an exact idea of the relative efficiency of some of those salts may be had from a study of Tables 5 and 6, which show the separate action of sodium and that of a few acid radicals.

SPECIFIC EFFECT OF RADICALS

The statement has already been made that the effect of a sodium salt represents the combined effect of the sodium and of the acid radical. That statement is based on experiments of which the following is an illustration:

To several flasks, each containing 750 c. c. of skim milk, varying portions of different salts were added. The salt portions were such that the same weight of sodium was introduced into each. All of the flasks received similar treatments except that different flasks received different acid radicals. Any variations in the resulting effects may be ascribed therefore to that source. The results are summarized in Table 5.

TABLE 5.—*Effect of the acid radicals of salts upon the body of artificial buttermilk*

[The quantities added correspond to 0.5 gm. of sodium per flask]

Name of salt	Number of grams of salt per flask	Quantity of undesirable lumps (mgm.)	Viscosity in seconds
Sodium mucate	2. 7838	457	35. 0
Disodium phosphate	3. 8939	494	30. 8
Trisodium citrate	2. 5881	291	24. 6
Sodium acetate	2. 9565	138	19 4
Sodium chloride	1. 2717	198	18. 0
Disodium sulphate	1. 5435	144	16. 2

In the experiment reported in Table 6 monosodium salts were added so that the 0.23 gm. of sodium added to each flask corresponded to equal concentrations of the acid radicals.

TABLE 6.—*Effect of acid radicals on the body of artificial buttermilk*

[The quantities added correspond to 0.23 gm. of sodium per flask]

Name of salt	Moles per flask	Quantity of undesirable lumps (mgm.)	Viscosity in seconds
Sodium chloride	0. 01	11	23. 9
Sodium acetate	. 01	15	20. 4
Monosodium phosphate	. 01	102	

In order to observe the effect of sodium in the experiment, salts of a polybasic acid like phosphoric acid containing equal fractions of the phosphoric radical were used. Thus the flask receiving the disodium salt automatically received twice as much sodium as the flask receiving, in the monosodium salt, an equal concentration of the phosphoric radical. The acid radicals of both salts may be

considered equivalent qualitatively, just as they are quantitatively, for Loeb (*10*) has shown that divalent and polyvalent radicals of acids like phosphoric, citric, and tartaric, react with proteins very much as do monovalent radicals. Table 7 illustrates the results obtained in the experiments with monosodium and disodium phosphate.

TABLE 7.—*Effect of sodium on the body of artificial buttermilk*

Name of salt	Moles per flask	Quantity of undesirable lumps (mgm.)	Viscosity in seconds
Monosodium phosphate	0.01	168.0	18.4
Disodium phosphate	.01	104.0	19.2

A careful examination of the tables recording the effect of sodium salts on the quality of buttermilk shows clearly the beneficial effect of sodium salts in reducing the lumpiness and the viscosity of buttermilk. In fact, the body may be weakened at will and may finally reach a point where the formation of a coagulum ceases, resulting in a product slightly thicker than milk but having the characteristic flavor of buttermilk.

It is evident also that sodium salts have individual action resulting from their sodium and acid constituents. It is possible to divide the different salts studied into groups having a similar, but not identical action. The chlorides, the acetates, and the sulphates, for instance, give a very soft, jellylike coagulum, reduce the tendency to whey off, and weaken the body of buttermilk extensively. The phosphates and the mucates increase the cohesion of the particles and have little effect in reducing lumpiness in the lower concentrations. While the citrates, tartrates, and malates hold a middle position, the carbonates form a class apart because of the very weak, gassy curd which they produce.

It must be understood that this classification of sodium salts is correct only in a general way, and that the individual salts within the same group vary widely in their action and the characteristic curd they produce.

THE CHEMICAL PROCESSES INVOLVED WHEN A SODIUM SALT IS ADDED TO MILK AND WHEN MILK IS HEATED

ACTION OF SODIUM SALTS

It is not the object of this work to present a general discussion of the views held by different investigators regarding the chemical and physicochemical constitution of milk. The view held by Van Slyke and Bosworth (*13*) and prevalent in this country is that casein in milk is in the form of tetracalcium or tricalcium caseinate. When a sodium salt is added to normal milk, a reaction of the following type probably takes place: Calcium caseinate + sodium chloride ⇌ calcium sodium caseinate + calcium chloride.

The addition of $CaCl_2$ or of NaCl makes the reaction proceed in one direction or the other. Thus enough $CaCl_2$ may be added to precipitate the casein as calcium caseinate, while an excess of NaCl

results in the formation of the very soluble sodium caseinate or of calcium sodium caseinate, and an increase in soluble calcium which is replaced by sodium (*3, 13*).

Laqueur and Sackur (*7*) explain the increase in viscosity observed when sodium hydroxide is added to a casein solution, by the formation of the highly dissociated sodium caseinate. Beyond a certain point, a further addition of the alkali causes a drop in viscosity. Laqueur and Sackur attribute that drop in viscosity to the depressing effect of the sodium on the dissociation of sodium caseinate. Pauli (*11*), accepting their view, goes further, advancing the hypothesis that each protein ion is hydrated; that is, each individual protein ion is surrounded by a shell of water, while the molecules are not hydrated. According to Pauli, hydration of the ions accounts for the higher viscosity of the more dissociated salts.

The studies made by various investigators thus indicate that a sodium salt added to normal milk results in the formation of the soluble and highly dissociated sodium caseinate or of a calcium-sodium caseinate characterized, according to Bosworth (*3*), by the softness and the fine texture of their curds. This highly dissociated sodium caseinate results in a more uniform coagulum which, when broken, produces a smooth, lump-free body. Moreover, sodium caseinate, being very soluble, does not coagulate readily; a softer curd and an increased capacity to absorb water therefore result.

In addition to their beneficial effects on milk by the formation of sodium caseinate, sodium salts contribute to some extent in at least four other ways to an improvement in the body of buttermilk.

They increase the solvent action of the serum. Lindet (*8, 9*) has demonstrated that the casein of milk is soluble in the serum constituents, and has shown that the presence of the so-called "lactalbumin" in milk is due entirely to the solvent action of the serum on milk caseins. An addition of sodium citrate to milk in the concentration of 4 parts per 1,000 increased its soluble proteins from 6.12 gm. to 8.94 gm. per liter. Consequently, it is very likely that some of the added salts exert a solvent action, thus increasing the softness of the curd. A simple calculation shows that a very small amount of sodium is sufficient to replace even the whole calcium of the caseinate molecule; the relatively large excess of sodium salt added is necessary, however, to make the reversible reaction shown above proceed in the desired direction; and when the salt used is capable of dissolving casein, that excess undoubtedly exerts a marked solvent action on the curd. Table 8 shows the results obtained by Lindet (*8*) with an artificial serum of the indicated concentration.

TABLE 8.—*Solubility of casein in the constituents of the serum*

Constituent	Concentration (parts per 1,000)	Quantity of casein dissolved (gm. per liter)
Lactose	50.0	0.270
Sodium chloride	2.0	.605
Sodium citrate	.8	.945
Sodium phosphate	.5	1.636

Adding a salt to milk probably results in the formation of some free acid after coagulation. The free acid formed by increasing the hydrogen-ion concentration increases at the same time the solubility of the casein-acid salt, resulting in a softer curd. Different acids produce different solvent actions. Chick and Martin (*4*) have observed the formation of some free acid in solutions of pure protein-acid salts. They attribute it to hydrolysis of those compounds as follows:

$$Protein - Hcl \rightleftharpoons Protein + H^+ + Cl^-$$

While this may be true in the case of sour milk, it is necessary to assume previous union between casein and free acid liberated from the added salt. The possibility of such liberation is clear if it is understood that in addition to the casein-acid salt, sour milk contains several other salts in solution, some citric acid, and an increasing amount of lactic acid. A part of those acids is in combination with casein; the rest remain in the serum either in the free state or united to the bases present in the milk. Salts and acids of the serum, on the other hand, are in a state of dynamic equilibrium which may be represented, in the case of sodium chloride, as follows:

$$CH_3.CHOH.COOH + NaCl \rightleftharpoons HCl + CH_3.CHOH.COONA$$
$$\text{lactic acid} \qquad\qquad \text{sodium lactate}$$
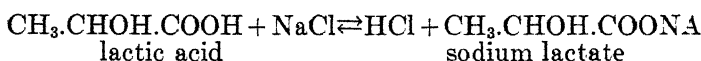
or expressing it in another form, as follows:

$$Hcl = K\frac{[CH_3.CHOH.COOH][NaCl]}{[CH_3.CHOH.COONa]}$$

It is thus shown that the amount of free acid formed varies directly with the amount of the salt added and the concentration of lactic acid. To such a change is attributed the alteration ln the flavor of buttermilk even when a tasteless salt has been added to the milk.

The salts added as well as the free acid formed may affect the cohesion of particles. The strong cohesion that the addition of phosphates to milk causes in the resulting coagulum has already been pointed out. Loeb (*10*) found that acetic acid markedly decreases the cohesion between the particles of gelatin. This agrees with the present study which shows that the addition of acetates to milk results in the formation of a smooth curd relatively free from lumps.

Some of the salts added have a strong buffer action which, by slowing down the rate of increase in hydrogen-ion concentration, tends to produce a slow coagulation. It must be understood, nevertheless, that in a complex mixture like milk, this is a minor factor. Table 9 gives the buffer values of different salts in various concentrations as found by Loeb (*10*).

TABLE 9.—*The buffer action of salts on milk*

Name of salt	Molecular concentrations			
	0	M/64	M/16	M/4
Sodium chloride	3.0	3.00	3.00	3.00
Sodium sulphate	3.0	3.05	3.20	3.35
Monosodium phosphate	3.0	3.20	3.40	3.50
Monosodium tartrate	3.0	3.45	3.55	
Monosodium citrate	3.0	3.60	3.75	
Sodium acetate	3.0	4.60		

ACTION OF HEAT

Several important changes in milk have been attributed to heat. Emphasis was laid in the past on the purely chemical ones, while the more recent investigations stress the changes that take place in milk as a colloidal system. Among the important chemical changes that take place are the precipitation of a part of the soluble milk proteins and also of the phosphates and the citrates in the form of saturated calcium salts, and the inactivation or destruction of milk enzymes. Most heated milks are coagulated with difficulty by rennet extract. How the above changes result in marked improvement in the body of buttermilk is hard to say. The bacterial factor, while very important, has been overestimated and is inadequate to explain the results obtained in this study. It is the writer's opinion that certain of the chemical changes promoted by heat are largely responsible for the improvement of the product, and that improvement by the addition of sodium salts and by heating are brought about largely through similar processes. As has been previously pointed out, the transformation of the sodium and calcium salts of casein to one another is a reversible change and proceeds in one direction or the other, depending on conditions. The change which takes place constantly in normal milk when sodium salt is added to it is illustrated by the formula,

$$\text{Calcium caseinate} + \text{sodium chloride} \rightleftharpoons \text{sodium caseinate} + \text{calcium chloride}$$
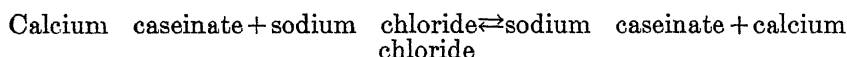
The addition of sodium chloride causes the reaction to proceed to the right while the addition of calcium chloride has the opposite effect. The same conditions are brought about by the removal of calcium chloride and of sodium chloride, respectively. When milk is heated, some of the citrates and phosphates are precipitated as the saturated calcium salts and a reduction in the concentration of soluble calcium salts takes place. That throws the above reaction to the right. A study of the relation between the improvement observed at different temperatures in the present study, and the decrease in the concentration of soluble calcium salts at the same temperatures, as determined by other investigators, revealed that they proceed in the same direction; although, due to other effects of heat, it was found that the improvement proceeds faster. The changes referred to are mainly of a colloidal nature. They are not very well understood, but investigations made with other proteins show that they tend to decrease the stability of milk. Chick and Martin (5) report that a solution of egg white which has been denatured by heat, precipitates at a considerable range of hydrogen-ion concentration, but that precipitation takes place more slowly as the concentration becomes greater and also less. Here again, the beneficial effect of slow coagulation is observed.

MISCELLANEOUS EXPERIMENTS

Starch and gelatin were added to milk in different concentrations and the effect on the resulting buttermilk was studied. The results obtained show that gelatin has practically no effect on the lump content of buttermilk. When starch in concentrations ranging from

0.1 to 0.5 per cent is added to the milk before heating and the milk is incubated at room temperature, the resulting buttermilk tends to increase in lumpiness and viscosity. When starch in concentrations of 0.2 per cent or above is added under the same conditions, the resulting buttermilk tends to have a starchy flavor.

Because of the sensitiveness of buttermilk to foreign flavors an attempt was made to improve its body by purely physical means. The product was run through a homogenizer at pressures of 2,500, 4,000, and 5,000 pounds, respectively. The results were surprisingly disappointing. Instead of the smooth, creamy, lump-free buttermilk expected, this test resulted in a liquid that separated on standing for a few minutes into two layers. The lower layer consisted of most of the serum from the buttermilk, and the top layer of a spongy, light, dry curd. Homogenization, while reducing the size of particles, failed to reduce the curd to colloidal dimensions; the pressure and the mechanical breakage of the capillaries resulting from homogenization served to express most of the serum from the curd.

Homogenizing the milk at different temperatures and pressures was also tried, but no improvement resulted. The data secured from homogenizing milk are recorded in Table 10.

TABLE 10.—*Effect of homogenizing milk before souring on the quality of artificial buttermilk*

Flask No.	Treatment	Homogenizing temperature (°C)	Quantity of un-desirable lumps (mg.)	Viscosity in seconds
1	Homogenized	9	139	28 2
2	do	38	141	28. 2
3	do	38	136	27. 4
4	Not homogenized		130	27. 3
5	do		159	28. 0

CONCLUSIONS

The influence of bacterial factors on the quality of artificial buttermilk has been emphasized to the exclusion of chemical factors. This study was conducted in order to determine whether or not the quality of buttermilk can be influenced by chemical means.

It was found that quality may be improved by the addition of various sodium salts to the milk before souring, but that such treatment resulted in altering the flavor of the product.

Heating milk at 100° C. produced marked improvement over heating at 82°. This difference, however, was not due entirely to the elimination of harmful microorganisms.

An attempt to study the mechanism of the chemical changes involved in making buttermilk led to evidences suggesting that adding a sodium salt to milk and heating it would improve the quality of the resulting buttermilk by similar processes. The tendency of artificial buttermilk to whey off when incubated at rather high temperatures was found to be due largely to physical forces.

Homogenizing artificial buttermilk resulted in the separation of serum; homogenizing milk before souring produced no beneficial effects.

Under the conditions of the experiments discussed in this study, substances like gelatin and starch produced no improvement in quality of buttermilk.

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NET-ENERGY VALUES OF CORN SILAGE, SOY-BEAN HAY, ALFALFA HAY, AND OATS [1]

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INTRODUCTION

The object of the respiration calorimetric experiments herein recorded was to gain a wider knowledge of the net-energy values of feeds for cattle.

The experimental subjects were three Aberdeen Angus steers each weighing about 800 pounds. Steer No. 254 was calved on February 8, No. 36 on October 10, and No. 47 on August 29—all in the year 1924—and the experimental program was conducted between October, 1926, and May, 1927. The ages of these steers, therefore, during the experiments were as follows: No. 254, 32 months and 19 days to 36 months and 18 days; No. 36, 27 months and 16 days to 29 months and 29 days; and No. 47, 26 months and 1 day to 32 months and 8 days. During the five months preceding these experiments the steers had been on good pasture without grain feed; they were, therefore, in good flesh; that is, about half fat.

The plan of the experiments contemplated (1) the determination of the fasting heat production of the experimental subjects as measures of the maintenance requirement of energy, and (2) the complete accounting for the energy of the rations used, each at two levels of intake, maintenance and production. The schedule of these experiments comprises Table 1.

FEEDS AND RATIONS

During periods 1, 7, 9, and 14 the subjects received no feed; and during the remaining 10 periods they received corn silage, soy-bean hay, or alfalfa hay, either alone or with a grain supplement.

The steers, however, refused to eat without waste the rations which the writers wished to study in quantities sufficient for the production of any considerable body increase. In the course of the season's experiments, many changes of plan were imposed by this action of the experimental subjects; the contemplated periods on full feed were found to be impracticable; corn stover, which had been included in the list of feeds to be studied, had to be dropped from the experimental program; and the amounts of the rations finally fed, as determined by the willingness of the steers to eat them without waste, were in general about such as were required to maintain energy equilibrium.

The planes of nutrition in the 10 feeding periods are shown by the balances of energy in Table 1. The amount of the smallest ration fed in period 3 was arbitrarily fixed at one-half that eaten in period 2. Otherwise the steers received about as much as they would take without any waste.

TABLE 1.—*Experimental periods, rations, and live weights of animals, experiment No. 237*

Period No.	Steer No.	Preliminary feeding period on experimental ration	Total digestion periods	Calorimeter periods	Daily rations				Balance of energy	Average live weight
					Roughage		Concentrate			
					Kind	Amount fed daily	Kind	Amount fed daily		
						Kgm		*Kgm.*	*Cals.*	*Kgm.*
1	254	Fasting		Oct. 27-29	Corn silage	12.0	Cottonseed meal	0.450	Fast.	357.2
2	47	Oct. 30-Nov. 4	Nov. 5-19	Nov. 17-19	...do	6.0	...do	.225	1,490 4	359.8
3	254	Nov. 15-20	Nov. 21-Dec. 3	Dec. 1-3	...do	12.0			-1,866 4	345.3
4	47	Nov. 22-29	Nov. 30-Dec. 17	Dec. 15-17	...do	3.8			434 0	361.5
5	254	Dec. 10-13	Dec. 14-Dec. 31	Dec. 29-31	Soy-bean hay	4.5			1,392.3	347.0
6	47	Dec. 28-Jan. 2	Jan. 3-Jan. 14	Jan. 12-14	...do				103 7	357.4
7	36	Fasting		Jan. 26-29					Fust.	334 0
8	254	Jan. 27-Jan. 31	Feb. 1-19	Feb. 9-11	Corn silage	3 9	Oats	2,600	389.8	339.7
9	36	Fasting		Feb. 23-26					Fast.	333.7
10	254	Feb. 11-21	Feb. 22-Mar. 11	Mar. 9-11	Alfalfa hay	4.6			-923.0	329.5
11	36	Mar. 10-14	Mar. 15-26	Mar. 23-25	...do	5.0	Oats	2.000	-753 1	355.0
12	47	Mar. 14-21	Mar. 22-Apr. 8	Apr. 6-8	...do	2.0	...do	2.130	327.5	318.5
13	36	Mar. 27-Apr. 4	Apr. 5-22	Apr. 20-22	...do	2.13			655.9	347.0
14	47	Fasting		May 4-7					Fast.	344.5

The difficulties encountered and the compromises necessitated in the feeding of the steers were due to the fact that whereas the method of experimentation required that the feed be consumed without waste, it is customary for cattle receiving considerable amounts of roughage to do some picking and choosing, and the rations which the writers wished to study were less palatable than those on which the steers had been reared.

In periods 2 and 3 a very small amount of cottonseed meal was fed with the corn silage in an effort to stimulate feed consumption, to avoid nitrogen loss, and to prevent an anticipated laxative tendency of the silage. The effort to stimulate feed consumption was not successful; the nitrogen balance was practically an equilibrium in both periods (Table 3); and corn silage by itself, in the largest quantity fed, 12 kgm. (26.2 pounds), proved not to be definitely laxative.

In periods 5 and 6 soy-bean hay was fed alone. In an effort to obtain a highly palatable hay without woody stems, which the animals would reject, the beans were cut when in bloom—somewhat earlier than is customary—but even so, and in spite of the fact that the hay was very successfully cured, the animals could not be induced to eat any considerable quantity. In period 5 steer No. 254 lost 1,392 Calories per day—a comparatively small amount—while in period 6 steer No. 47 was almost exactly in energy balance.

In periods 8, 12, and 13 ground oats were fed with either corn silage or alfalfa hay, and thus it became possible, by a comparison of the results with those obtained with the roughages alone, to determine the net-energy value of the oats.

In periods 10 and 11 alfalfa hay was fed alone. The hay was of good quality, but the animals would not eat quite enough of it, by itself, to maintain energy equilibrium.

The net-energy values determined in this series of experiments, therefore, apply not to production but much more nearly—and without considerable error—to maintenance.

EXPERIMENTAL PROCEDURE

The duration of the preliminary feeding on the kind of feed to be used in the subsequent experimental period was in no case less than 10 days, while the length of the preliminary period on the particular amount of feed to be used in the subsequent experiment was as indicated in Table 1, column 3; the length of digestion periods (normally 18 days, but sometimes necessarily abbreviated) is given in column 4; and the duration of the periods of continuous calorimeter experimentation was as indicated in column 5, all dates being inclusive.

The calorimeter periods in which the animals received feed were all 3 days in length, while the fasting calorimeter periods were 3 or 4 days in length—3 in period 1 and 4 in periods 7, 9, and 14.

In the preparation of the steers for the determination of the fasting katabolism roughage was withheld for a day, and grain for the latter half of the same day. Then each steer was given two half-pound doses of physic, two hours apart, the physic being composed of the following components in the proportions indicated: Sodium sulphate 12 ounces, capsicum 2 ounces, ginger 2 ounces, and gamboge 1 ounce. This physic was administered in the morning of a given day and the measurement of the heat production of fast (except in period 1) began on the second morning afterward, the first day of

measurement, therefore, being the third day of fast. In period 1 the first day of the heat measurement was the eighth day of fast.

Salt was fed in all feeding periods to the amount of 30 gm. (15 gm. per feed) per day. An effort was made to have the fasting steers take a nutrient salt mixture to prevent loss of salts from the body and to stimulate water drinking, but the steers did not care for it. Fasting cattle have no appetite for salt, and comparatively little desire for water.

All feeds were sampled for analysis in the usual manner at the time they were weighed out for feeding. In the case of corn silage this involved daily sampling, two 2-quart bottles being taken each day. These daily portions were kept in a cold-storage room, at a temperature below 0° F., and were combined and sampled for analysis at the end of the experimental period. The feeds, except the corn silage, were analyzed from the fresh condition.

The analysis of the silage, except for the determination of nitrogen and carbon, was made on material dried in a steam-heated air oven at a temperature of about 60° C. Nitrogen was determined in the fresh material. Carbon was determined in a combustion furnace on fresh material, cut up by a draw-cut meat chopper, and also in the dried material. The energy determination on the dried material was corrected for the loss of carbon in drying by adding 9.4 Calories for each gram of carbon lost, on the assumption that this loss was carbohydrate.

The feces were sampled daily, the combined aliquots being kept at a temperature below 0° F., without preservative, until the end of the current experimental period, when they were sampled for analysis.

The feces were analyzed from the air-oven-dried material, except that nitrogen and carbon were also determined in the fresh product; dry matter was directly determined by means of the vacuum desiccator; and energy was determined in the vacuum-dried material. The energy as determined was corrected for the slight loss of carbon and nitrogen during drying.

Urines were sampled daily and preserved at about 34° F., with the addition of chloroform.

EXPERIMENTAL DATA

The digestibility of the rations is shown in Table 2. These data are not used in the determination of net energy, but are included because they are available, and for such interest as they may have for students and others who use such values.

There are a number of appreciable differences between these coefficients of digestibility and the average values compiled by Henry and, Morrison,[2] though most of these lie within the range of normal variation, as affected by the composition of the feeds and the individuality of the animal used as the experimental subject. The only notable difference is in the digestibility of the ether extract of the alfalfa hay, in regard to which the very low values found may be due to the low planes of nutrition prevailing and to the ether extract of the feces being largely of metabolic origin instead of being derived solely from the feed, as implied by the method of computation of digestibility.

The balances of matter and energy are recorded in Table 3. The computed heat production is derived by computation from the income

[2] HENRY, W. A., and MORRISON, F. B. FEEDS AND FEEDING. Ed. 18, unabridged, 770 p., illus. Madison, Wis. 1923.

and outgo of carbon, nitrogen, and energy; the observed heat production is the heat directly measured; and the balance of energy is the metabolizable energy minus the observed heat production.

TABLE 2.—*Digestibility of rations, experiment No. 237*

Period No	Animal No	Feeds and other items	Dry matter	Organic matter	Crude protein	Crude fiber	N-free extract	Ether extract	Carbon	Energy
			Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
2	47	Salt	30.0							
		Silage	3,607.0	3,411.8	294.0	835.7	2,190.5	91.6	1,702.0	16,473.0
		Cottonseed meal	411.7	381.3	191.9	39.5	120.0	29.9	197.5	2,051.8
		Total feed	4,049.0	3,793.1	485.9	875.2	2,310.5	121.5	1,899.5	18,524.8
		Feces	1,164.0	1,031.4	187.6	307.2	518.6	18.0	554.6	5,477.1
		Percentage digestibility	71.3	72.8	61.4	64.9	77.6	85.2	70.8	70.4
3	254	Salt	30.0							
		Silage	1,794.0	1,693.3	135.8	431.6	1,080.0	45.9	861.5	8,412.2
		Cottonseed meal	205.8	190.8	96.0	21.6	58.2	15.0	99.1	1,033.1
		Total feed	2,029.8	1,884.0	231.8	453.2	1,138.2	60.9	960.6	9,445.3
		Feces	562.9	488.7	95.8	140.5	242.5	9.9	266.1	2,727.4
		Percentage digestibility	72.3	74.1	58.7	69.0	78.7	83.8	72.3	71.1
4	47	Salt	30.0							
		Silage	3,370.6	3,197.3	275.3	785.3	2,047.5	89.2	1,610.3	15,526.1
		Feces	1,004.0	909.5	137.7	276.7	481.5	13.7	500.3	4,921.7
		Percentage digestibility	70.5	71.6	50.0	64.8	76.5	84.6	68.9	68.3
5	254	Salt	30.0							
		Soy-bean hay	3,337.5	3,054.2	664.5	1,050.7	1,275.0	64.0	1,537.9	15,099.5
		Feces	1,261.6	1,082.7	165.7	505.1	368.9	43.0	606.9	6,032.4
		Percentage digestibility	62.2	64.6	75.1	51.9	71.1	32.7	60.5	60.1
6	47	Salt	30.0							
		Soy-bean hay	3,979.4	3,653.6	772.1	1,252.5	1,561.2	67.9	1,837.8	18,128.0
		Feces	1,489.7	1,314.6	194.1	610.9	458.7	50.9	713.9	7,031.3
		Percentage digestibility	62.6	64.0	74.9	51.2	70.6	25.0	61.2	61.2
8	254	Salt	30.0							
		Silage	1,125.5	1,065.4	91.2	272.4	670.5	31.3	541.4	5,239.5
		Oats	2,260.2	2,175.1	307.3	267.1	1,494.6	106.1	1,067.1	10,756.6
		Total feed	3,415.7	3,240.5	398.5	539.5	2,165.1	137.4	1,608.5	15,996.1
		Feces	1,081.5	978.2	129.5	310.9	526.5	11.3	511.1	5,120.2
		Percentage digestibility	68.3	69.8	67.5	42.4	75.7	91.8	68.2	68.0
10	36	Salt	30.0							
		Alfalfa hay	4,016.2	3,672.9	641.4	1,343.8	1,625.7	62.0	1,862.3	18,159.8
		Feces	1,780.5	1,538.4	227.9	730.6	513.4	66.6	877.2	8,610.9
		Percentage digestibility	56.0	58.1	64.5	45.6	68.4		52.9	52.6
11	47	Salt	30.0							
		Alfalfa hay	4,426.9	4,038.6	720.0	1,465.3	1,783.5	69.8	2,067.5	20,428.0
		Feces	1,968.6	1,688.0	246.8	808.7	567.3	65.3	990.8	9,774.3
		Percentage digestibility	55.8	58.2	65.7	44.8	68.2	6.5	52.1	52.2
12	36	Salt	30.0							
		Alfalfa hay	1,782.2	1,639.3	265.8	612.6	734.4	26.6	826.2	8,123.8
		Oats	1,753.4	1,691.3	244.3	199.0	1,163.1	84.9	826.6	8,332.8
		Total feed	3,565.6	3,330.6	510.0	811.6	1,897.5	111.5	1,652.7	16,456.6
		Feces	1,312.1	1,162.4	169.7	470.6	491.0	31.0	627.8	6,247.7
		Percentage digestibility	63.2	65.1	66.7	42.0	74.1	72.2	62.0	62.0
13	47	Salt	30.0							
		Alfalfa hay	1,899.6	1,745.5	287.4	657.2	773.4	27.5	826.2	8,546.6
		Oats	1,866.7	1,797.4	259.8	217.6	1,231.0	89.0	876.6	8,798.2
		Total feed	3,796.3	3,542.9	547.2	874.8	2,004.4	116.5	1,756.1	17,344.8
		Feces	1,278.0	1,131.4	152.5	474.9	473.2	30.8	610.1	6,103.6
		Percentage digestibility	66.3	68.1	72.1	45.7	76.4	73.5	65.3	64.8

The results of the experiments are based on the directly determined heat production, the computed values being used simply as controls.

The fasting heat production, which constitutes the measure of the maintenance requirement of energy, is given in Table 4. This measurement as now taken is quite acceptable, but an effort is being made to improve it by determining the exact treatment necessary entirely to free the alimentary tract from feed residues, and by measuring directly instead of computing the surface area.

It will be noted that the fasting data used were derived from periods 7, 9, and 14. The results of period 1 were not used since these represented days of fast 8, 9, and 10, whereas it was found that the average of the results from days 3, 4, and 5, seemed to represent an approximately true state of fast, and were preferred as derived from the

shortest period acceptable for the purpose. In period 14 the electric power was off during part of the first calorimeter day and on this account the results of only the last two days, representing the fourth and fifth days of fast, were used.

TABLE 3.—*Balance of matter and energy per head and day, experiment No. 237*

PERIOD 2, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
Corn silage	3,607.0	8,385.0	47.0	1,702.0	16,473.0
Cottonseed meal	412.0	38.4	30.7	197.5	2,051.8
Water		4,055.0			
Total	4,019.0	12,478.4	77.7	1,899.5	18,524.8
Outgo:					
Feces	1,164.0	4,196.0	30.0	554.6	5,477.1
Urine	201.3	4,092.0	39.2	82.9	*a* 842.5
Methane	110.3			82.5	1,471.8
Carbon dioxide	3,936.2			1,073.4	
Water vapor		5,444.5			
Metabolizable:					
Income minus urine, feces, and methane					10,733.4
Body balances:					
Fat	103.6			79.3	984.2
Protein	51.0		8.5	26.8	*a* 227.4
Energy					*b* 1,490.4
Water		−1,254.1			
Computed heat production					*c* 9,521.8
Observed heat production					9,243.0

PERIOD 3, STEER NO. 254

Income:					
Corn silage	1,794.0	4,202.7	21.7	861.5	8,412.2
Cottonseed meal	205.8	19.2	15.4	99.1	1,033.1
Water		2,937.0			
Total	1,999.8	7,158.9	37.1	960.6	9,445.3
Outgo:					
Feces	562.9	1,666.0	15.3	266.1	2,727.4
Urine	166.3	4,137.0	30.8	55.6	*a* 428.6
Methane	78.5			58.8	1,047.5
Carbon dioxide	2,766.6			754.4	
Water vapor		3,306.0			
Metabolizable:					
Income minus urine, feces, and methane					5,241.8
Body balances:					
Fat	−190.7			−145.9	−1,811.7
Protein	−54.0		−9.0	−28.4	*a* −240.7
Energy					*b* −1,866.4
Water		−1,950.1			
Computed heat production					*c* 7,294.2
Observed heat production					7,108.2

PERIOD 4, STEER NO. 47

Income:					
Corn silage	3,370.6	8,656.0	44.0	1,610.3	15,526.1
Water		1,037.0			
Total	3,370.6	9,693.0	44.0	1,610.3	15,526.1
Outgo:					
Feces	1,004.0	4,467.0	22.0	500.3	4,921.7
Urine	194.7	3,533.0	19.6	59.2	*a* 558.9
Methane	98.7			73.8	1,317.1
Carbon dioxide	3,325.6			906.9	
Water vapor		4,793.3			
Metabolizable:					
Income minus urine, feces, and methane					8,728.4
Body balances:					
Fat	81.7			62.5	776.2
Protein	14.4		2.4	7.6	*a* 64.2
Energy					*b* 434.0
Water		−3,100.3			
Computed heat production					*c* 7,888.0
Observed heat production					8,290.4

a Corrected to nitrogen equilibrium.
b Metabolizable energy minus observed heat production.
c The computed heat is obtained by subtracting algebraically the gains of energy from the metabolizable.

TABLE 3.—*Balance of matter and energy per head and day, experiment No. 23;*—Continued

PERIOD 5, STEER NO. 254

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Cals.*
Income·					
Soy-bean hay	3,337.5	462.0	106.3	1,537.9	15,099.7
Water		11,013.0			
Total	3,337.5	11,475.0	106.3	1,537.9	15,099.5
Outgo;					
Feces	1,261.6	4,238.0	26.5	606.9	6,032.7
Urine	209.0	4,141.0	71.8	94.7	*a* 967.0
Methane	84.9			63.6	1,132.9
Carbon dioxide	3,176.7			866.3	
Water vapor		4,992.7			
Metabolizable:					
Income minus urine, feces, and methane					6,966.9
Body balances.					
Fat	−155.3			−118.8	−1,475.4
Protein	48.0		8.0	25.2	*a* 214.0
Energy					*b* 1,392.3
Water		1,896.7			
Computed heat production					*c* 8,228.3
Observed heat production					8,359.2

PERIOD 6, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Soy-bean hay	3,979.4	521.3	123.5	1,837.8	18,128.0
Water		10,742.0			
Total	3,979.4	11,263.3	123.5	1,837.8	18,128.0
Outgo·					
Feces	1,489.7	5,577.0	31.1	713.9	7,031.3
Urine	232.7	5,134.0	83.4	104.5	*a* 1,060.0
Methane	103.2			77.2	1,377.1
Carbon dioxide	3,371.8			919.5	
Water vapor		5,103.6			
Metabolizable:					
Income minus urine, feces, and methane					8,659.6
Body balances·					
Fat	−7.5			−5.7	−71.3
Protein	54.0		9.0	28.4	*a* 240.7
Energy					*b* 103.7
Water		−4,551.3			
Computed heat production					*c* 8,490.2
Observed heat production					8,555.9

PERIOD 8, STEER NO. 254

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income·					
Corn silage	1,125.5	2,777.0	14.6	541.4	5,239.5
Oats	2,260.2	352.7	49.2	1,067.1	10,756.6
Water		6,145.0			
Total	3,385.7	9,274.7	63.8	1,608.5	15,996.1
Outgo:					
Feces	1,081.5	4,390.0	20.7	511.1	5,120.2
Urine	159.7	3,215.0	33.2	58.1	*a* 660.0
Methane	93.4			69.9	1,246.3
Carbon dioxide	3,480.4			949.1	
Water vapor		5,212.5			
Metabolizable:					
Income minus urine, feces, and methane					8,969.6
Body balances:					
Fat	−14.2			−10.9	−134.9
Protein	59.4		9.9	31.2	*a* 264.8
Energy					*b* 389.8
Water		−3,542.8			
Computed heat production					*c* 8,839.7
Observed heat production					8,579.8

a Corrected to nitrogen equilibrium.
b Metabolizable energy minus observed heat production.
c The computed heat is obtained by subtracting algebraically the gains of energy from the metabolizable.

TABLE 3.—*Balance of matter and energy per head and day, experiment No. 237—*
Continued

PERIOD 10, STEER NO 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Cals.*
Income					
Alfalfa hay	4,016.2	584.3	102.6	1,862.3	18,159.8
Water		10,707.0			
Total	4,016.2	11,291.3	102.6	1,862.3	18,159.8
Outgo:					
Feces	1,780.5	7,417.0	36.5	877.2	8,610.9
Urine	252.3	3,433.0	59.3	94.6	a 1,013.2
Methane	76.9			57.4	1,026.2
Carbon dioxide	3,297.6			899.3	
Water vapor		4,701.6			
Metabolizable:					
Income minus urine, feces, and methane					7,509.5
Body balances.					
Fat	-114.6			-87.7	-1,088.7
Protein	40.8		6.8	21.4	a 181.9
Energy					b -923.0
Water		-4,260.3			
Computed heat production					c 8,416.3
Observed heat production					8,432.5

PERIOD 11, STEER NO 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Alfalfa hay	4,426.9	579.0	115.2	2,067.5	20,428.0
Water		11,920.0			
Total	4,426.9	12,499.0	115.2	2,067.5	20,428.0
Outgo:					
Feces	1,968.6	6,274.0	39.5	990.8	9,774.3
Urine	297.0	4,594.0	71.1	103.8	a 1,099.1
Methane	91.5			68.5	1,221.0
Carbon dioxide	3,611.9			985.0	
Water vapor		4,936.8			
Metabolizable:					
Income minus urine, feces, and methane					8,333.6
Body balances.					
Fat	-124.3			-95.1	-1,180.9
Protein	27.6		4.6	14.5	a 123.0
Energy					b -753.1
Water		-3,205.8			
Computed heat production					c 9,391.5
Observed heat production					9,086.7

PERIOD 12, STEER NO. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Alfalfa hay	1,788.2	221.3	42.5	826.2	8,123.8
Oats	1,753.4	217.0	39.1	826.6	8,332.8
Water		10,082.0			
Total	3,541.6	10,550.3	81.6	1,652.8	16,456.6
Outgo:					
Feces	1,312.1	4,219.0	27.2	627.8	6,247.7
Urine	294.7	3,567.0	48.4	68.0	a 774.8
Methane	97.9			73.3	1,306.4
Carbon dioxide	3,188.0			869.4	
Water vapor		3,725.6			
Metabolizable:					
Income minus urine, feces, and methane					8,127.7
Body balances:					
Fat	-6.0			-4.6	-57.0
Protein	36.0		6.0	18.9	a 160.5
Energy					b 327.5
Water		-961.3			
Computed heat production					c 8,024.2
Observed heat production					7,800.2

a Corrected to nitrogen equilibrium.
b Metabolizable energy minus observed heat production.
c The computed heat is obtained by subtracting algebraically the gains of energy from the metabolizable.

TABLE 3.—*Balance of matter and energy per head and day, experiment No. 237*—
Continued

PERIOD 13, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Gals.*
Income					
Alfalfa hay	1,899. 6	237. 7	46. 0	879. 5	8, 516. 6
Oats	1,866. 7	265. 0	41. 6	876. 6	8, 798. 2
Water		10,880 0			
Total	3,766 3	11,382 7	87. 6	1, 756. 1	17, 314. 8
Outgo:					
Feces	1,278. 0	3, 755. 7	24. 4	610. 1	6, 103. 6
Urine	203. 7	3, 537. 7	54. 6	75. 0	a 845. 0
Methane	103. 1			77. 1	1, 375. 8
Carbon dioxide	3,403. 7			928. 2	
Water vapor		3,776. 5			
Metabolizable:					
Income minus urine, feces, and methane					9, 020. 4
Body balances.					
Fat	50 5			38. 6	479. 8
Protein	51. 6		8. 6	27. 1	a 230. 0
Energy					b 655. 9
Water		312. 8			
Computed heat production					c 8, 310. 6
Observed heat production					8, 364. 5

a Corrected to nitrogen equilibrium.
b Metabolizable energy minus observed heat production.
c The computed heat is obtained by subtracting algebraically the gains of energy from the metabolizable.

TABLE 4.—*Daily fasting katabolism of steers, experiment No. 237*

Period No.	Steer No.	Day of fast	Body surface a	Heat production Per head	Heat production Per square meter of body surface
			Sq. m.	*Cals.*	*Cals.*
		Third	4. 15	6, 465. 6	1, 558. 0
		Fourth	4. 13	5, 920. 1	1, 433. 4
7	36	Fifth	4. 11	5, 830. 5	1, 418. 6
		Average		6, 072 1	1, 470. 0
		Third	4. 19	6, 516. 8	1, 555. 3
		Fourth	4. 17	6, 323. 6	1, 516. 5
9	254	Fifth	4. 15	6, 137. 2	1, 478. 8
		Average		6, 325. 9	1, 516. 9
		Fourth	4. 26	6, 663. 8	1, 564. 3
14	47	Fifth	4. 24	6, 403. 2	1, 510. 2
		Average		6, 533. 5	1, 537. 3

a Computed by Moulton's formula, 0.1186 $W^{\frac{2}{3}}$, in which W, the empty live weight, is the actual live weight times 0.9. See MOULTON, C. R. UNITS OF REFERENCE FOR BASAL METABOLISM AND THEIR INTERRELATIONS. Jour. Biol. Chem. 24: 299–320, illus. 1916.

The final results of the series of experiments are stated in Table 5.
The net energy for maintenance, per head, was computed from the
fasting heat production per square meter of body surface (Table 4)
by correction for the difference between the weight of the animal
during the fasting and the feeding experiments (Table 1).

The data regarding heat production were as directly determined
(Table 3); the total heat increments were derived by subtracting the

net energy requirements (per head) for maintenance from the corresponding figures for heat production; and the heat increments per kilogram of dry matter of feed were obtained by dividing the total heat increments by the kilograms of dry matter of feed eaten (Table 5).

The metabolizable energy per kilogram of dry matter was computed from the total metabolizable energy, as in Table 3, and the amount of dry matter of feed eaten; the net energy (for maintenance) per kilogram of dry matter is the similar figure for metabolizable energy minus the heat increment; and the utilization of the metabolizable energy is the percentage of the metabolizable energy which is net.

TABLE 5.—*Computation of net-energy values for maintenance, experiment No. 237*

Period No.	Animal No.	Dry matter (in kilograms) of feeds eaten	Total dry matter of feeds eaten	Live weight	Body surface	Net energy for maintenance — Per square meter body surface	Net energy for maintenance — Per head	Heat production	Heat increment — Total	Heat increment — Per kilogram dry matter of feed	Metabolizable energy per kilogram dry matter	Net energy per kilogram dry matter	Utilization of metabolizable energy
			Kgm.	*Kgm.*	*Sq. m.*	*Cals*	*Cals.*	*Cals*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Per cent*
2	47	Silage. 3.607 Cottonseed meal, 0.412	4.019	359.8	4.40	1,537.3	6,764.1	9,419.4	2,655.3	661	2,671	2,010	75.3
3	254	Silage, 1,794. Cottonseed meal, 0.206	2,000	345.3	4.29	1516.9	6,507.5	7,129.7	622.2	311	2,621	2,310	88.1
2	47	Silage ª									2,651	1,959	73.9
3	254	do.ª									2,596	2,294	88.4
4	47	do.	3.371	361.5	4.41	1,537.3	6,779.5	8,434.2	1,654.7	491	2,589	2,098	81.0
5	254	Soy bean hay	3,338	347.0	4.30	1,516.9	6,522.7	8,474.4	1,951.7	585	2,087	1,502	72.0
6	47	do.	3.979	357.4	4.38	1,537.3	6,733.4	8,673.0	1,939.6	487	2,176	1,689	77.6
8	254	Silage, 1.126. Oats, 2,260.	3.386	339.7	4.24	1,516.9	6,431.7	8,634.3	2,202.6	651	2,649	1,998	75.4
10	36	Alfalfa hay	4.016	329.5	4.16	1,470.0	6,115.2	8,515.7	2,400.5	598	1,870	1,272	68.0
11	47	do.	4.427	355.0	4.36	1,537.3	6,702.6	9,159.6	2,457.0	555	1,882	1,327	70.5
12	36	Alfalfa hay, 1.788. Oats, 1,753.	3.541	318.5	4.07	1,470.0	5,982.9	7,937.5	1,954.6	552	2,295	1,743	75.9
13	47	Alfalfa hay, 1,900. Oats, 1,867.	3.767	347.0	4.30	1,537.3	6,610.4	8,486.9	1,876.5	498	2,395	1,897	79.2
12	36	Oats ª							885.4	505	2,729	2,224	81.5
13	47	do.ª							822.0	440	2,916	2,476	84.9

ª See text for method of computation.

The results of the first two feeding periods, 2 and 3, in which a small proportion of cottonseed meal was fed, were used for the computation of the net-energy value (for maintenance) of corn silage by employing Kellner and Köhler's production value for cottonseed meal, as quoted by Armsby,[3] increased by the use of the ratio (1 : 0.761), determined at this institute,[4] between the utilization of energy for maintenance and production. While the basis of this computation is imperfect, the average of the two results (2,126.5 calories) is only about 1 per cent greater than the more directly determined value (2,098 Calories) found in period 4. It is therefore of little moment whether the three values for silage are averaged, or whether the third figure (2,098 Calories) is accepted. The result of period 2 (1,959 Calories) agrees fairly well with the more directly determined value (2,098 Calories) found in period 4. The relatively low heat increment and high net-energy values obtained in period 3

[3] ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 660. New York. 1917.
[4] FORBES, E. B., FRIES, J. A., BRAMAN, W. W., and KRISS, M. THE RELATIVE UTILIZATION OF FEED ENERGY FOR MAINTENANCE, BODY INCREASE, AND MILK PRODUCTION OF CATTLE. Jour. Agr. Research 33: 483–492. 1926.

are quite likely due to the very low plane of nutrition, and can hardly be accepted as applying to maintenance. All things considered, it seems best to regard the directly determined figure (2,098 Calories) as the provisional net-energy value of corn silage, for maintenance.

The two values for net energy (for maintenance) of soy-bean hay (1,502 and 1,689 Calories) agree only fairly well, but are provisionally acceptable.

The net-energy value of silage and oats, as determined in period 8, may be utilized to yield a net-energy value of either component of the ration by the use of the value for silage, as found in period 4, or the value for oats, as found in periods 12 and 13. Inasmuch, however, as values so determined would involve possible error through the comparison of results obtained with different experimental subjects, and inasmuch as the values computed by this imperfect procedure differ materially from the other values reported, derived from results in which there is no confusion of individuality of experimental subjects, no use is made of the results of period 8 at this time.

The values determined for alfalfa hay in periods 10 and 11 (1,272 and 1,327 Calories) agree fairly well, the larger being about 4 per cent in excess of the smaller; and the average may be accepted as the provisional net-energy value of alfalfa hay for maintenance.

. By the use of these values and the results of periods 12 and 13, in which alfalfa and oats were fed, the values found for net-energy of oats, for maintenance, were 2,224 and 2,476 Calories per kilogram of dry matter. The higher of these values is about 11 per cent in excess of the lower—which leaves appreciable improvement to be accomplished in future determinations.

In connection with the net-energy values presented in Table 5, it is well to note that the duplicate determinations for soy-bean hay (periods 5 and 6), alfalfa hay (periods 10 and 11), alfalfa hay and oats (periods 12 and 13), and oats alone (computed by difference) were obtained on different animals, and that in each case steer No. 47 gave the higher value for net and metabolizable energy, and the lower value for heat increment.

It is also striking that the heat increments are much less affected by the kind of feed than are the metabolizable energy values. This accounts to a large extent for the markedly different percentages of utilization of the metabolizable energy in the last column of Table 5. These data show that the metabolizable energy of concentrates is much more largely utilizable than is that of roughages. Net energy, therefore, is much more significant and consistent than would be metabolizable energy as a measure of the energy value of a feed in nutrition.

SUMMARY

The following net-energy values, per kilogram of dry matter of feeds, for the maintenance of approximately 800-pound 2 to 3 year old beef steers are submitted, these values being determined by direct calorimetry, using the heat production during fast as the measure of the maintenance requirement of net energy: Corn silage, 2,098 Calories; soy-bean hay, 1,502 and 1,689 Calories; alfalfa hay, 1,272 and 1,327 Calories; and ground oats, 2,224 and 2,476 Calories.

The heat increments caused by the feeds were found to be much more nearly proportional to the dry matter than to the metabolizable

energy of the feed; and the percentages of utilization of the metabolizable energy were found to be much affected by the nature of the feed, especially as to whether a roughage or a concentrate; from which it follows that the net energy of a feed is much more significant and consistent than is metabolizable energy (feed minus excreta) as a measure of the energy value of a feed in nutrition.

The maintenance requirements of net energy of the three steers used as subjects were 1,470, 1,517, and 1,537 Calories, respectively, per square meter of body surface, and 1,818, 1,896, and 1,896 Calories, respectively, per 100 kgm. (S26, 859, and 859 Calories per 100 pounds) of live weight.

O

RUN-OFF FROM SMALL AGRICULTURAL AREAS[1]

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INTRODUCTION

The purpose of the experiments herein recorded was to determine the rates of run-off from small agricultural areas. The results are especially applicable to the design of open-ditch drainage systems, terrace systems, small diversion ditches, storm-water sewers draining areas consisting partly of farm lands, tile drains with surface inlets, and road culverts where characteristics of the watersheds and other conditions are similar to those herein described.

An area of 112 acres with well-defined divides and adequate drainage channels, situated about 4½ miles southeast of Jackson, Madison County, Tenn., was chosen as the site for these experiments. Nearly all of the area was included in a farm owned by M. N. Murchison. The experiments consisted in making rainfall and run-off measurements on six watersheds ranging in area from 1¼ acres to 112 acres.

GENERAL DESCRIPTION OF WATERSHEDS

Figure 1 shows the size and shape of the several watershed areas and the physical characteristics that affect run-off, such as timber, gullies, land slopes, and the arrangement of the main drainage channels and hillside ditches. The data for this map were obtained from a carefully made stadia and transit survey. Particular care was taken to locate accurately the divides between the watersheds so that the areas could be correctly determined. As may be judged from the contours, the topography of the tract is quite hilly; in fact, it is typical of the steepest lands under cultivation in the county. The soil is of the type known as Lexington silt loam, described by the United States Bureau of Soils [2] as follows:

The Lexington silt loam, to a depth of about 12 inches, is a gray or yellowish-gray mellow silt loam. In the virgin state the surface 2 or 3 inches is usually slightly darker in color than the rest of the profile, owing to the accumulation of a small amount of organic matter. Where the type is under cultivation it is also usually somewhat darker in color and more friable. The subsoil to a depth of 36 inches consists either of a compact, plastic silt loam, slightly heavier than the soil, or of a silty clay varying from yellow to brown in color or sometimes tinged with red. Very often the subsoil in the lower depths is mottled yellow, brown, and gray and has a mealy texture. At an average depth of 3 feet the orange sand occurs. * * *

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 9
May 1, 1927
Key No. D-18

51054—27——1   (797)

Watershed boundaries........ +--+--+
Edge of oak timber..........
Barbed wire fences...........
Ditches over 3 feet deep......
Ditches less than 3 feet deep.....
Height above datum...........—120—

SCALE OF FEET

100 50 0   500   1000

FIG. 1.—Watershed areas on the Murchison farm near Jackson, Tenn.   51054—27. (Face p. 798.)

The topography of the Lexington silt loam is moderately rolling to hilly. The hilly portion consists mainly of long, winding ridges, forming the watersheds between the streams, and is sparsely settled and but little cultivated. * * * *

The topography gives practically all of the type excellent drainage. The character of the deep subsoil is such that considerable of the soil moisture is removed by seepage. The growing crops are seriously injured by droughts if of extended duration, but they are seldom damaged by wet weather, as are those on the lowlands. Springs are more numerous than in any other soil. * * *

About 24 per cent of the area is covered with timber. The cleared lands have been devoted to the cultivation of cotton for many years with very little protection against erosion. As a result most of the top soil on the steeper slopes has been washed away by the heavy rains and deposited over the bottom areas or carried off the watershed. The subsoil over the steeper slopes is exposed in many places and does not possess the fertility necessary to produce a profitable crop. As may be seen from Figure 1, hillside ditches have been employed to some extent to check erosion, but they have not proved to be very successful.

In Table 1 are given some data pertaining to the characteristics of each of the six watersheds. In the second column is given the size of the watershed in acres. The fall of the channels at the gauging station, the average fall from the farthest point on the watershed to the gauging station, and the maximum fall found on any of the hillsides of the watershed are given in the next three columns. This information is of use in making a comparative study with other watersheds where it is desired to apply the results of these experiments. The distance from the farthest point on the watershed to the gauging station and the time required for water to travel this distance—known as the time of concentration for the watershed—are given in the next two columns. This distance is as measured along the watercourse from the gauging station and in a direct line from the upper end of the watercourse to the farthest point on the watershed. In the last column is given the percentage of the watershed area that is covered with timber. Timber on a watershed has a decided effect upon the rates and amounts of run-off, as will be brought out later in the discussion of the results.

TABLE 1.—*Characteristics of six watersheds on the Murchison Farm near Jackson, Tenn.*

Watershed No.	Area	Fall of channel at gauging station	Average fall from farthest point to gauging station	Maximum fall	Distance from farthest point to gauging station	Time of concentration	Percentage in timber
	Acres	*Feet per 100 feet*	*Feet per 100 feet*	*Feet per 100 feet*	*Feet*	*Minimum*	
1	20.70	2.4	4.89	40	1,220	5	14.0
3	49.20	2.2	3.99	64	2,152	10	24.7
4	15.70	3.5	5.44	32	1,418	7	38.9
5	112.00	1.0	2.74	64	3,933	17	23.9
6	1.25	----------	9.78	33	359	1½	0.0
7	2.79	----------	8.53	39	672	3	55.5

METHODS AND APPARATUS

RUN–OFF

Run-off is that portion of the rainfall which finds its way through the ground or over the surface to drainage channels. In general the amount of run-off is equal to the rainfall minus the evaporation and

seepage into the substrata. The rate of run-off for small agricultural areas or storm-sewer areas usually is expressed in inches per hour or in cubic feet per second per acre. The rate of run-off depends upon the size and shape of the watershed, surface slopes, nature and amount of vegetation character of the soil as regards permeability, saturated condition of the soil due to previous rains, arrangement and character of drainage channels, evaporation, storage and underground conditions, and the duration and intensity of the rainfall. However, of these factors affecting the rate of run-off, evaporation is practically negligible for small watersheds.

The maximum rate of run-off from any watershed area for a given intensity of rainfall occurs when all parts of the area are contributing to the flow. That part of the watershed nearest the outlet must still be contributing to the flow when the water from the most remote point on the watershed reaches the outlet. To fulfill this condition the rain must continue as long as is required for the water to travel from the most remote point of the watershed to the outlet or gauging station. This interval is called the time of concentration for the watershed. The maximum rate of run-off therefore would result from a rainfall of maximum uniform intensity continuing for a time equal to or exceeding the time of concentration.

What is known as the rational method of computing the maximum rate of run-off from a watershed is based upon the foregoing principle and is expressed by the following equation:

$$Q = C\ I\ A$$

Where Q = Rate of run-off in cubic feet per second.
C = Run-off coefficient or coefficient of imperviousness, representing the ratio of the rate of run-off to the rate of rainfall.
I = Rainfall intensity in cubic feet per second per acre, or approximately in inches per hour.
A = The watershed area in acres.

The rational method is rapidly supplanting the use of empirical formulas that have largely been depended upon in computing storm water flow but that do not make provision for the various factors affecting run-off.

In these investigations the values for Q, I, and A were ascertained by field measurements and the value of C was determined by solving the equation, $Q = C\ I\ A$.

FIELD MEASUREMENTS

The field measurements relating to rainfall and run-off consisted of gauging the discharges of the various channels and measuring the rates and amounts of the rainfall during the period of the investigations.

DISCHARGE MEASUREMENTS

The flow of water in four of the channels was ascertained from measurements made of the velocities and cross-sectional areas, and in two of the channels by means of weir boxes.

Measurements of velocity were made with a rod current meter at intervals of 1 foot across the channel and in most cases at top, middle, and bottom depths. For some of the gaugings, however, the velocity was measured at the surface or at 0.6 foot depth. In the three-point method the mean velocity was obtained by taking one-sixth of the

sum of the velocity at the top, four times the velocity at the middle, and the velocity at the bottom. The mean velocity for each 1-foot section was then determined by averaging the velocities in the verticals on each side of the section, and the discharge for the section was obtained by multiplying this mean velocity by the area of the section between the verticals. Discharge measurements were made for various stages in the channel, except that very few high-stage measurements were made owing to the difficulty of reaching the stations at the time of the crest flow, which usually lasted but a few minutes. The gaugings of the channels on the smaller watersheds
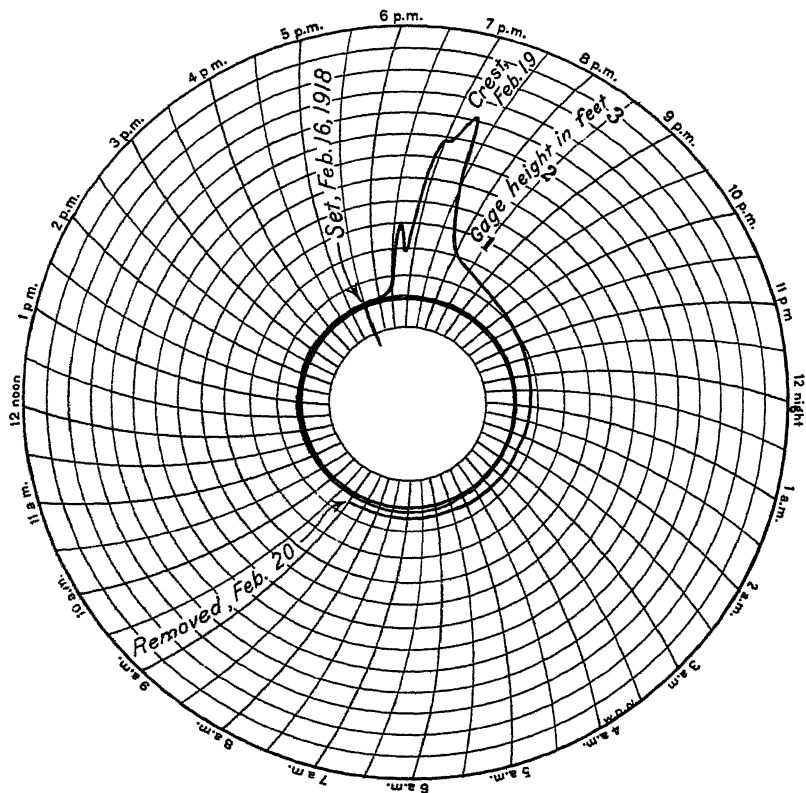
Fig. 2.—Recorder chart showing record of gauge heights for period February 16–20, 1918, at gauging station No. 5

were made during the heaviest rainfall, since the water in the channels subsided rapidly when the rain ceased. The discharge of the chan-nels for the highest stages that occurred were computed by the Kutter and Chezy formulas, using the value n computed for the highest stage in the channel for which a gaging was made. The discharges, obtained from gauging and computations, were used to construct discharge curves for the channels, from which the discharge capacity of a channel could be determined for any stage.

Continuous records of the stages in the channels were obtained with automatic water-stage registers. Recorders were used that covered a range of 5 feet in the water level. A record of the water

stages in one of the channels obtained with the instrument used is shown in Figure 2. The amount and time of all fluctuations in the water surface are recorded on a circular chart. The chart is revolved by a clock at the rate of 1 revolution in 24 hours, and a pivoted pen which moves up and down—corresponding to changes in the water level—records the water stage continuously.

The run-off from two of the smallest watershed areas was measured by means of weir boxes. These boxes were constructed in accordance with specifications prepared by V. M. Cone.[3] The weir boxes were made 20 feet long, 2 feet wide at the lower end, 2½ feet wide at the upper end, and 1½ feet deep. The bottom of each box was made level and flush with the bottom of the ditch at the upstream end. An enlargement in both depth and width of the ditch was made a short distance above the upper end of the box to serve as a catch basin to remove a part of the load of silt carried by the run-off water. This basin was found practically filled with silt and sand after every heavy rain and at the heaviest rates of precipitation considerable sand was carried through and deposited in the box. A steel weir plate was installed at the downstream end of the box, the weir being 1 foot wide and the crest flush with the bottom of the box so as to reduce to a minimum the accumulation of sand above the weir. The depth of the water in the box was measured with a recorder placed 6 feet upstream from the weir. The accumulation of silt and sand in the weir box and in the vertical flume inclosing the compensator of the recorder was the unavoidable cause of inaccuracy in some of the measurements. The following formula, derived from experimental data and contained in the article by Cone,[3] above referred to, was used in the determination of the weir discharges:

$$Q = (3.83 - 0.07L) \ Lh \ (1.52 + 0.01L).$$

Where L = length of weir crest in feet,
h = head on weir crest in feet,
Q = discharge in cubic feet per second.

RAINFALL MEASUREMENTS

Measurements of the rainfall were made with a tipping-bucket rain gauge at the location shown in Figure 1. The rain is collected in a funnel 12 inches in diameter and is poured into a bucket with two compartments each holding 0.01 inch of rain. The bucket is so supported that as a compartment is filled it tips and discharges the rain water into the receptacle below, at the same time presenting the other compartment for filling. At each tip of the bucket an electrical contact is made which causes the recording pen to make a step on the chart. The chart is carried on a cylinder that is turned by a clock at the rate of 1 revolution in six hours. The mechanism that regulates the movement of the pen is such as to cause the pen to move five steps across the chart and five steps back. Record charts from this instrument of 11 of the most intense rains that occurred during the year 1918 are shown in Figures 3 and 4. Each division of time on the chart is equivalent to 5 minutes, and it is possible to estimate the time to the nearest minute. Tests of the instrument for heavy rates of precipitation showed that owing to the

³ CONE, V. M. A NEW IRRIGATION WEIR. Jour. Agr. Research 5 : 1127-1143, illus. 1916.

appreciable time required for the bucket to tip, a small quantity of water will flow into the bucket after it begins to tip. Tests accordingly were made to determine the amount lost in this manner for different rates of precipitation, and a correction was applied to the records of precipitation. A check on the total amount of rainfall recorded for each rain was obtained by direct measurement of the rain collected in the receptacle below the tipping bucket. The time
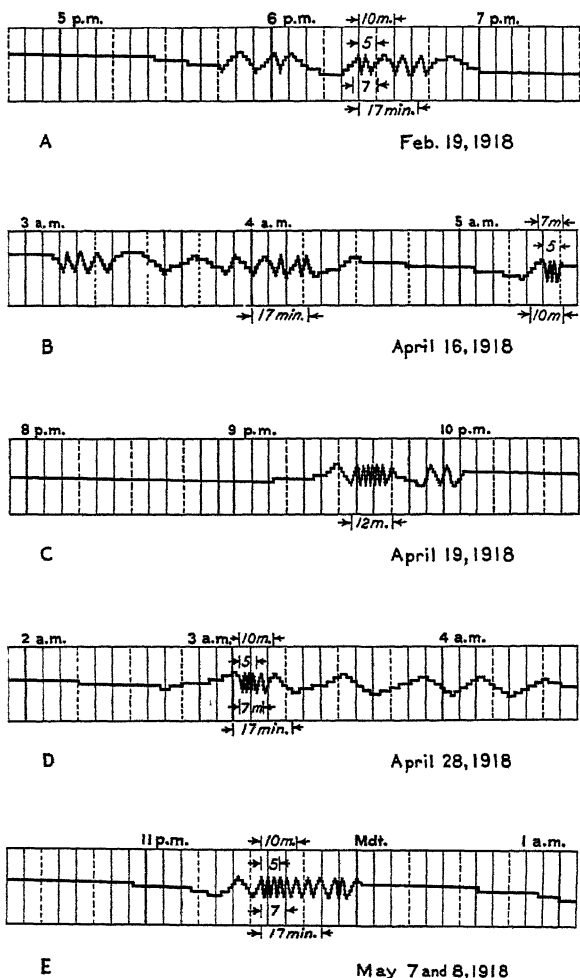
Fig. 3.—Precipitation on the Murchison farm near Jackson, Tenn., for selected storms

recorded by the instrument was checked with a watch at the time the chart was removed. An attendant living close by wound the clock and changed the charts at noon daily.

MEASUREMENTS OF RAINFALL AND RUN-OFF

The field measurements were first begun in March, 1917, and were continued throughout the year 1918. Data were secured for all rains

during this period, but since by far the most satisfactory results were obtained for the year 1918—during which time water-stage recorders particularly adapted to the needs of the experiments were used—it was not believed that the data for 1917 would add to the value of the results and conclusions. It was also decided to present the results only for the most intense rains, since the primary purpose of these experiments is to provide data on *maximum* rates of run-off.
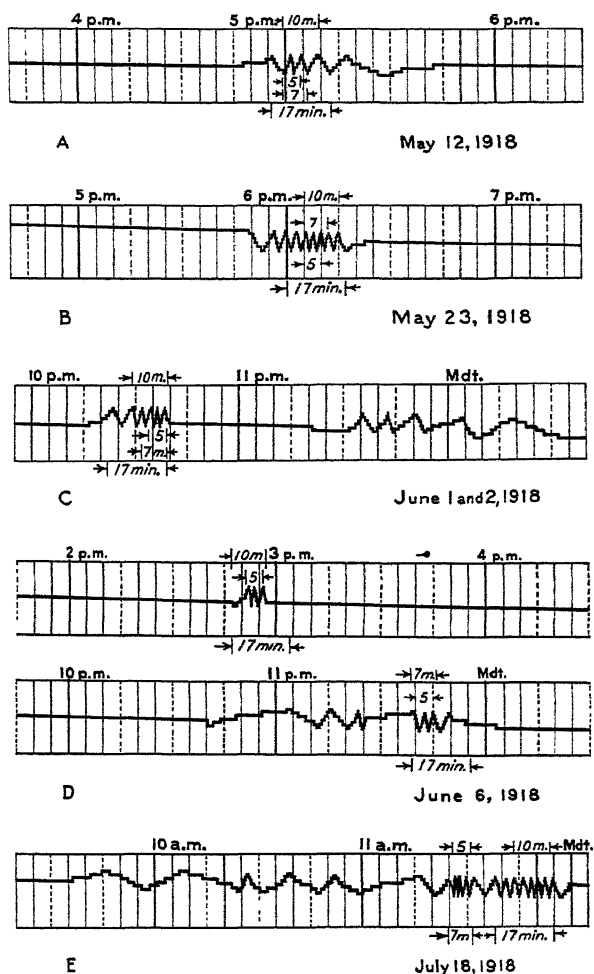
FIG. 4.—Precipitation on the Murchison farm near Jackson, Tenn., for selected storms

Measurements were contemplated on seven watershed areas, numbered from 1 to 7. (See fig. 1.) However, satisfactory data were not obtained for watershed No. 2. Owing to the "flashy" nature of the streams, which subsided as soon as the rains ended, there was not time to secure satisfactory measurements at all of the stations following each rain, and gauging station No. 2 was the last to receive attention.

TABLE 2.—*Measurements of rainfall and run-off for watershed No. 1; drainage area, 20.7 acres; time of concentration, 5 minutes*

Date of rain, 1918	Rainfall during time of concentration	Average rate of rainfall during time of concentration	Maximum rate of run-off	Coefficient of run-off (ratio of maximum rate of run-off to average rate of rainfall)	Rainfall prior to period taken as time of concentration	Previous rains over 0.08 inch		See Figure No.
						Date	Amount	
	Inch	*Inches per hour*	*Second-feet*	*Inches per hour*		*Inches*	*Inches*	
Feb. 19	0.20	2.40	20 0	0.96	0.40	0.39	Feb. 15 / Feb. 12 / Feb. 6	0.10 / .41 / .20	3, A
Apr. 16	.32	3.84	26.5	1.27	.33	1.21	Mar. 23 / Mar. 20 / Apr. 20	.23 / .13 / .82	3, B
Apr. 28	.37	4.44	38.0	1.82	.41	.09	Apr. 19 / Apr. 17 / Apr. 16	1 59 / .72 / 1.55	3, D
May 7	.30	3.60	28 5	1.37	.38	.18	Apr. 28 / Apr. 20 / Apr. 19	1.01 / .82 / 1.59	3, E
May 12	.16	1.92	16.5	.79	.41	.10	May 11 / May 8 / May 7	.11 / .45 / 1.19	4, A
May 23	.26	3.12	32.0	1.53	.49	.38	May 20 / May 18 / May 12	.09 / .13 / .55	4, B
June 1	.27	3.24	24.5	1.17	.36	.46	May 23 / May 20 / May 18	.92 / .09 / .13	4, C
June 6, 3 p. m.	.25	3.00	29.5	1.41	.47	.04	June 5 / June 2 / June 1	.76 / .19 / 1.13	4, D
June 6, 11 p. m.	.19	2.28	20.0	.96	.42	.72	June 5 / June 2 / June 1	.76 / .19 / 1.13	4, D
July 18	.35	4.20	44.5	2.13	.51	.76	July 17 / June 29 / June 28	.20 / .19 / .11	4, E

The results of the field measurements and computations for the several watersheds are given in Tables 2 to 7. The watersheds are referred to in the tables by number and the same numbers have been used in referring to the gauging stations of the respective watersheds. The drainage area or watershed area and the time of concentration for the watershed appear in the heading of each table. The time of concentration, as has been explained, is the time required for the water to flow from the farthest point on the watershed to the gauging station. This was determined by noting the time required for the water in the channel at the gauging station to rise from the low to the maximum stage as recorded by the water-stage recorders. This period varied to some extent for the different rains, depending upon the degree of saturation of the watershed at the occurrence of the rain that produced the maximum rate of run-off. Also the time of concentration would be less if the channel were partially filled with water when the rain of greatest intensity occurred, and would be less [for] rains of high intensity than for rains of less intensity. However, [ow]ing to the inability of the recording instruments to register the [tim]e with sufficient accuracy, the difference in the time of concen[tra]tion for the different rains on the same watershed could not be [sa]tisfactorily determined, and the time of concentration was taken [to] be the same for the different rains on the same watershed, with [th]e exception which will be referred to later.

TABLE 3.—*Measurements of rainfall and run-off for watershed No. 3; drainage area, 49.2 acres; time of concentration, 10 minutes*

Date of rain, 1918	Rainfall during time of concentration	Average rate of rainfall during time of concentration	Maximum rate of run-off		Coefficient of run-off (ratio of maximum rate of run-off to average rate of rainfall)	Rainfall prior to period taken as time of concentration	Previous rains over 0.08 inch		See Figure No.
							Date	Amount	
	Inch	*Inches per hour*	*Second-feet*	*Inches per hour*		*Inches*		*Inches*	
Feb. 19	0 24	1 44	33 0	0 67	0 47	0.39	Feb. 15	0.10	
							Feb. 12	.41	3, A
							Feb. 6	.20	
Apr. 16	.34	2 04	42 5	.86	.42	1.17	Mar. 23	.23	3, B
							Mar. 20	.13	
							Apr. 20	.82	
Apr. 28	.52	3.12	72.0	1.45	.46	.09	Apr. 19	1.59	3, D
							Apr. 17	.72	
							Apr. 16	1.55	
							Apr. 28	1.01	
May 7	.54	3.24	58 0	1.17	.36	.18	Apr. 20	.82	3, E
							Apr. 19	1.59	
							May 11	.11	
May 12	.28	1.68	26.0	.52	.31	.10	May 8	.45	4, A
							May 7	1.19	
							May 20	.09	
May 23	.46	2.76	68.0	1 37	.50	.38	May 18	.13	4, B
							May 12	.55	
							May 23	92	
June 1	.44	2 64	39.0	.79	30	.28	May 20	.09	4, C
							May 18	.13	
							June 5	.76	
June 6, 3 p. m.	.34	2 04	42.5	.86	.42	.00	June 2	.19	4, D
							June 1	1.13	
							July 17	.20	
July 18	.50	3 00	80.0	1.61	.54	1.41	June 29	.19	4, E
							June 28	.11	

TABLE 4.—*Measurements of rainfall and run-off for watershed No. 4; drainage area, 15.7 acres; time of concentration, 7 minutes*

Date of rain, 1918	Rainfall during time of concentration	Average rate of rainfall during time of concentration	Maximum rate of run-off		Coefficient of run-off (ratio of maximum rate of run-off to average rate of rainfall)	Rainfall prior to period taken as time of concentration	Previous rains over 0.08 inch		See Figure No.
							Date	Amount	
	Inch	*Inches per hour*	*Second-feet*	*Inches per hour*		*Inches*		*Inches*	
Feb. 19	0.22	1 89	6.5	0.41	0.22	0.37	Feb. 15	0.10	
							Feb. 12	.41	3, A
							Feb. 6	.20	
Apr. 16	.34	2.92	13.5	.85	.29	1.19	Mar. 23	.23	3, B
							Mar. 20	.13	
							Apr. 20	.82	
Apr. 28	.44	3.77	15.0	.95	.25	.09	Apr. 19	1.59	3, D
							Apr. 17	.72	
							Apr. 16	1.55	
							Apr. 28	1.01	
May 7	.41	3.51	15.5	.98	.28	.18	Apr. 20	.82	3, E
							Apr. 19	1.59	
							May 11	.11	
May 12	.22	1.89	9.0	.57	.30	.10	May 8	.45	4, A
							May 7	1.19	
							May 20	.09	
May 23	.33	2.83	15.5	.98	.35	.38	May 18	.13	4, B
							May 12	.55	
							May 23	.92	
June 1	.35	3.00	13 5	.85	.28	.38	May 20	.09	4, C
							May 18	.13	
							June 5	.76	
June 6, 11 p. m.	.25	2 14	9 5	.60	.28	.69	June 2	.19	4, D
							June 1	1.13	
							July 17	.20	
July 18	.41	3.51	25 5	1.61	.46	.71	June 29	.19	4, E
							June 28	.11	

TABLE 5.—*Measurements of rainfall and run-off for watershed No. 5; drainage area, 112 acres; time of concentration, 17 minutes*

Date of rain, 1918	Rainfall during time of concentration	Average rate of rainfall during time of concentration	Maximum rate of run-off	Maximum rate of run-off	Coefficient of run-off (ratio of maximum rate of run-off to average rate of rainfall)	Rainfall prior to period taken as time of concentration	Previous rains over 0.08 inch — Date	Amount	Total rainfall for storm period	Total run-off for storm period	Total run-off in per cent of total rainfall	See Figure No.
	Inch	*Inches per hour*	*Sec-ond-feet*	*Inches per hour*		*Inches*			*Inches*	*Inch*		
Feb. 19	0.40	1.41	68.0	0.60	0.43	0.39	Feb. 15 Feb. 12 Feb. 6	0.10 .41 .20	0.95	0.48	50.5	3, A and 9, A
Apr. 16	.40	1.41	69.0	.61	.43	.64	Mar. 23 Mar. 20 Apr. 17	.23 .13 .72	1.55	.57	36.8	3, B and 9, B
Apr. 19	.70	a 3.50	333.5	2.95	.85	.12	Apr. 16	1.55	1.08	.92	85.2	3, C and 10, A
Apr. 28	.58	2.05	90.0	.80	39	.07	Apr. 20 Apr. 19 Apr. 17 Apr. 16	.82 1.59 .72 1.55	------	------	------	3, D
May 7	.75	2.65	85.5	.76	.29	.18	Apr. 28 Apr. 20 Apr. 19	1.01 .82 1.59	1.31	.39	29.8	3, E and 10, B
May 12	.37	1.31	39.4	.35	.27	.05	May 11 May 8 May 7	.11 .45 1.19	.52	.18	34.6	4, A and 10, C
May 23	.67	2.36	120.5	1.07	.45	.20	May 20 May 18 May 12	.09 .13 .55	.92	.30	32.6	4, B and 11, A
June 1	.59	2.08	70.0	.62	.30	.16	May 23 May 20 May 18	.92 .09 .13	1.14	.37	32.5	4, C and 11, B
June 6, 3 p. m.	.34	b 1.20	56.0	.50	.42	.00	June 5 June 2 June 1	.76 .19 1.13	.33	.17	51.5	4, D and 11, C
June 6, 11 p. m.	.34	1.20	66.0	.58	.48	.69	June 5 June 2 June 1	.76 .19 1.13	.71	.35	49.3	4, D and 12, A
July 18	.76	2.68	124.5	1.10	.41	1.21	July 17 June 29 June 28	.20 .19 .11	2.17	.63	29.0	4, E and 12, B

a Time of concentration for this rain taken as 12 minutes.   b Rain fell in 10 minutes.

In the first column of Tables 2 to 7 are given the dates of the most intense rains that occurred during the year 1918. In column 2 is given, for each rain, the amount of rain that fell during the time of concentration, from which were computed the values given in column 3, which are the average rates of rainfall, in inches per hour, during the time of concentration. In columns 4 and 5 are shown the maximum rates of run-off in cubic feet per second and in inches per hour. Columns 7, 8, and 9 contain rainfall data, from which an idea can be formed as to the degree of saturation of the watershed. In column 7 is given the amount of rain that fell at the beginning of the storm—that is, prior to the period taken as the time of concentration. Columns 8 and 9 show the dates and amounts of previous rains each of which exceeded 0.08 inch, that contributed to the saturation of the watershed. In column 6 are given the coefficients of run-off or the ratios of the average rates of rainfall during the time of concentration to the maximum rates of run-off. These are the coefficients C for use in the formula $Q = CIA$, which is employed in the rational method

TABLE 6.—*Measurements of rainfall and run-off for watershed No. 6; drainage area, 1.25 acres; time of concentration, 1½ minutes*

Date of rain, 1918	Rainfall during time of concentration	Average rate of rainfall during time of concentration	Maximum rate of run-off	Coefficient of run-off (ratio of maximum rate of run-off to average rate of rainfall)	Rainfall prior to period taken as time of concentration	Previous rains over 0.08 inch — Date	Amount	See Figure No.
	Inch	*Inches per hour*	*Second-feet*	*Inches per hour*		*Inches*		*Inches*
Feb. 19	0.07	2.8	2.04	1.62	0.58	0.39	Feb. 15; Feb. 12; Feb. 6	0.10; .41; .20	3, A
Apr. 16	.13	5.2	3.09	2.45	.47	1.23	Mar. 23; Mar. 20; Apr. 20	.23; .13; .82	3, B
Apr. 28	.13	5.2	3.88	3.08	.59	.09	Apr. 19; Apr. 17; Apr. 16; Apr. 28	1.59; .72; 1.55; 1.01	3, D
May 7	.10	4.0	2.27	1.80	.45	.18	Apr. 20; Apr. 19; May 11	.82; 1.59; .11	3, E
May 12	.07	2.8	.92	.73	.26	.11	May 8; May 7	.45; 1.19	4, A
May 23	.10	4.0	----	----	----	----			4, B
June 1	.10	4.0	1.76	1.40	.35	.50	May 23; May 20; May 18	.92; .09; .13	4, C
June 6, 3 p. m.	.10	4.0	1.38	1.10	.28	.06	June 5; June 2; June 1	.76; .19; 1.13	4, D
June 6, 11 p. m.	.07	2.8	1.51	1.20	.43	.76	June 5; June 2; June 1	.76; .19; 1.13	4, D
July 18	.14	5.6	3.36	2.67	.48	.76	July 17; June 29; June 28	.20; .19; .11	4, E

TABLE 7.—*Measurements of rainfall and run-off for watershed No. 7, drainage area, 2.79 acres; time of concentration, 3 minutes*

Date of rain, 1918	Rainfall during time of concentration	Average rate of rainfall during time of concentration	Maximum rate of run-off	Coefficient of run-off (ratio of maximum rate of run-off to average rate of rainfall)	Rainfall prior to period taken as time of concentration	Previous rains over 0.08 inch — Date	Amount	See Figure No.
	Inch	*Inches per hour*	*Second-feet*	*Inches per hour*		*Inches*		*Inches*
Apr. 16	0.24	4.8	3.20	1.14	0.24	1.23	Mar. 23; Mar. 20; Apr. 20	0.23; .13; .82	3, B
Apr. 28	.26	5.2	5.03	1.79	.34	.09	Apr. 19; Apr. 17; Apr. 16; Apr. 28	1.59; .72; 1.55; 1.01	3, D
May 7	.18	3.6	2.93	1.04	.29	.18	Apr. 20; Apr. 19; May 11	.82; 1.59; .11	3, E
May 12	.12	2.4	1.18	.42	.18	.11	May 8; May 7	.45; 1.19	4, A
May 23	.15	3.0	2.42	.86	.29	.38	May 20; May 18; May 12	.09; .13; .55	4, B
June 1	.17	3.4	2.42	.86	.25	.50	May 23; May 20; May 18	.92; .09; .13	4, C
June 6, 3 p. m.	.17	3.4	1.81	.64	.19	.04	June 5; June 2; June 1	.76; .19; 1.13	4, D
June 6, 11 p. m.	.12	2.4	1.85	.66	.28	.76	June 5; June 2; June 1	.76; .19; 1.13	4, D
July 18	.24	4.8	4.35	1.55	.32	.76	July 17; June 29; June 28	.20; .19; .11	4, E

of computing storm water flow. In column 10 of Tables 2, 3, 4, 6, and 7, and in column 13 of Table 5 are given references to the rainfall charts for each of the rains. These charts convey an idea as to the uniformity of the rate of rainfall during periods of intensity.

In columns 10, 11, and 12 of Table 5 are given, for each storm, the total amount of rain, the total amount of run-off, and the percentage of the rainfall that ran off. These data are not given for the smaller watersheds, since their streams are so flashy that the recorders were unable to register with accuracy the changes in the water level.

In Figure 5 is plotted the daily precipitation for the year 1918. The day was taken as beginning and ending at midnight; hence the total amount of rainfall for each rain as given in Table 5 may or may not correspond to the total rainfall given in Figure 5 for the period midnight to midnight, since, for instance, a rain may have begun slightly before midnight and continued into the next day. Such a rain would be recorded in Table 5 as occurring on the day the maximum rate of run-off occurred. Furthermore, where more than one rain
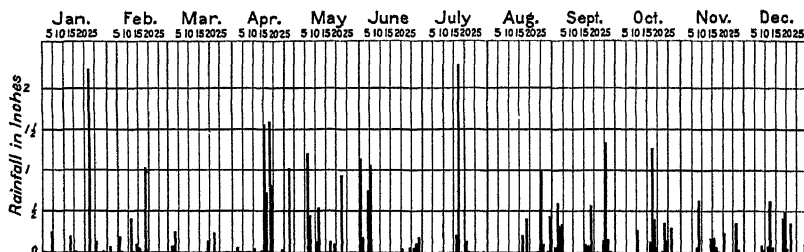
Fig. 5.—Record of daily precipitation from midnight to midnight on the Murchison farm for the year 1918

occurred between midnight and midnight (as on June 6) the total for each rain is recorded separately in the table. Figure 5 readily indicates the probable condition of the soil due to prior rains.

WATERSHED NO. 1

Watershed No. 1 is fan-shaped and contains 20.7 acres, 14 per cent of which is covered with timber. As may be seen from Figure 1, the timber consists of four scattered patches. The cleared portion of this watershed has been cultivated for many years, cotton being the principal crop. Figure 6, A, is a view of the watershed taken from the point marked *a* in Figure 1, and Figure 6, B, is a view taken from the rain-gauge station. Numerous deep gullies exist in the cleared area. Unsuccessful efforts have been made to check erosion by the use of hillside ditches. Over a large part of the cleared area there are bare places incapable of producing a crop, due to erosion. During the period of the investigation cotton was growing on a small part of the watershed near the gauging station; the remainder of the land lay idle.

The distance from the farthest point on the watershed to the gauging station is 1,220 feet, and the average fall is 4.89 feet per 100 feet, the fall of the channel at the gauging station is 2.4 feet per 100 feet, and the maximum fall on the steepest hillside of the watershed is 40 feet per 100 feet. Owing to the small percentage of timbered area
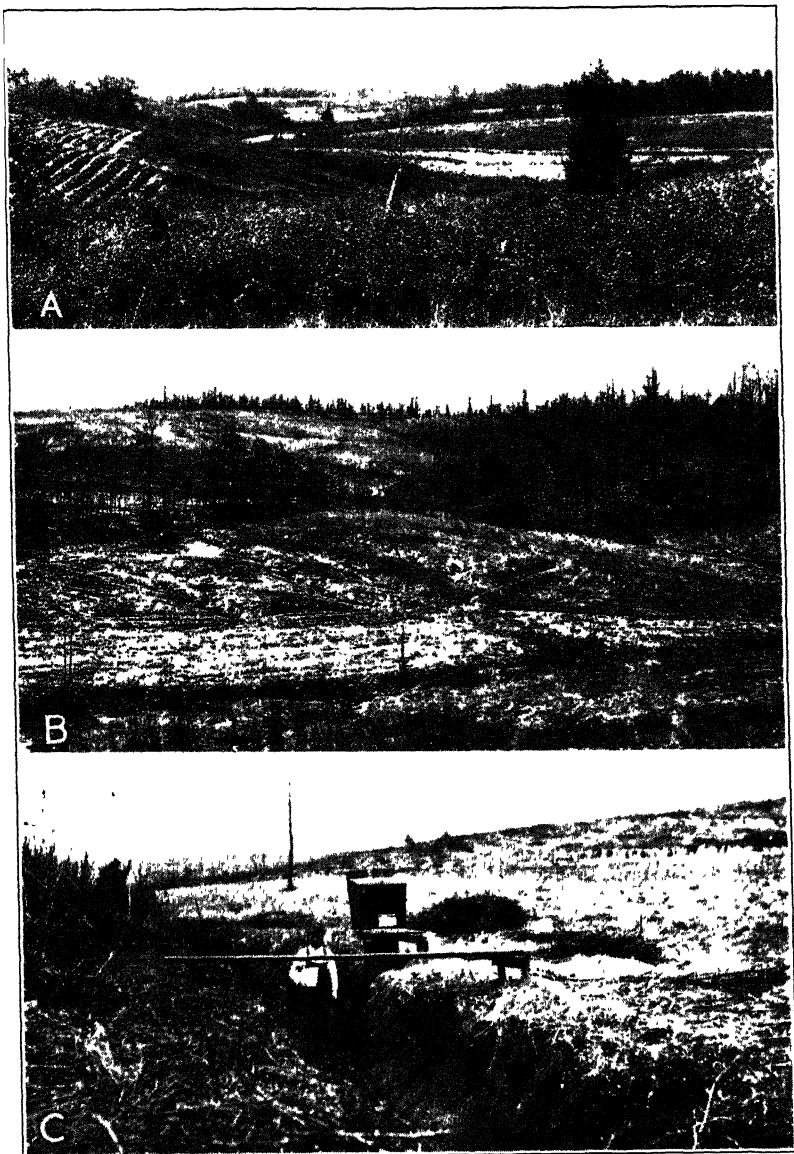
FIG. 6.—A, watershed No. 1 on Murchison Farm, taken from a point marked (*a*) in Figure 1; B, watershed No. 1, taken from rain-gauge station (gauging station No. 1 can be seen at the right); C, channel and gauging station for watershed No 1

and the numerous gullies which permit a large part of the watershed to drain quickly, the time of concentration for this watershed was found to be small—about 5 minutes—and the rates of run-off comparatively large.

A view of the channel and gauging station is shown in Figure 6, C. This is typical of the gauging stations on the other channels. The gauging bridge consists of a 2-inch plank laid across at right angles to the channel. Figure 7 shows a cross section of the channel at the gauging station. The highest stage in the channel, which occurred on April 19, 1918, and the highest stage resulting from the 10 storms given in Table 2—which stage occurred on July 18, 1918—are shown on the cross section. The rates of rainfall and run-off for these 10 storms are given in Table 2. The highest average rates of rainfall of five minutes duration for the 10 storms given in the table were 4.44 and 4.20 inches per hour and occurred on April 28 and July 18,
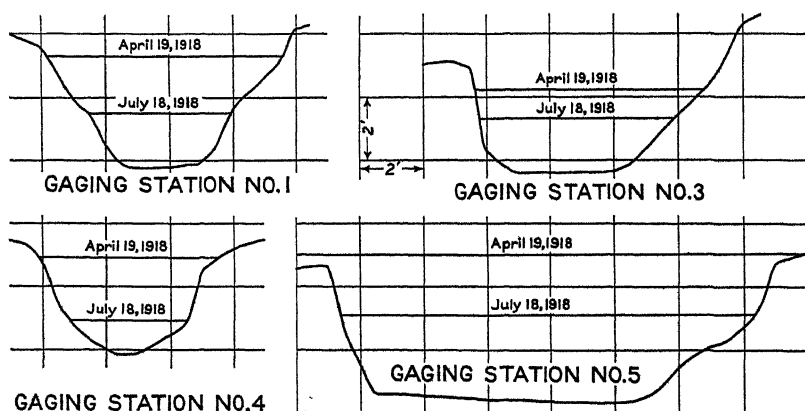
FIG. 7.—Cross sections of channels at gauging stations

respectively. The maximum rates of run-off were 38 and 44.5 cubic feet per second, or 1.82 and 2.13 inches per hour, respectively. It will be noted that the greater rate of run-off occurred for the smaller rate of rainfall. Doubtless this was due largely to the fact that the rain of July 18 was of more uniform intensity for the five-minute period than was the rain of April 28, and also to the difference in the degree of saturation of the soil prior to the intense rates of rainfall. Other factors being the same, one would expect a greater rate of run-off in the spring when there are no growing crops and only scanty vegetation, than in the summer. However, in this case the effect of vegetation was offset by the rain which fell shortly prior to the period of intense rainfall and saturated the vegetation and the ground surface. As may be seen from Table 2, the run-off coefficients for these rains are 0.41 and 0.51, respectively, and the coefficients range from 0.33 to 0.51 for the 10 rains given in the table. A study of the rainfall charts in Figures 3 and 4, and of the data on previous rains given in the table, will reveal in many cases the causes of the variations in the run-off coefficients.

WATERSHED NO. 3

Watershed No. 3 contains 49.2 acres, of which 24.7 per cent is covered with timber. It includes watersheds Nos. 1 and 2, and the
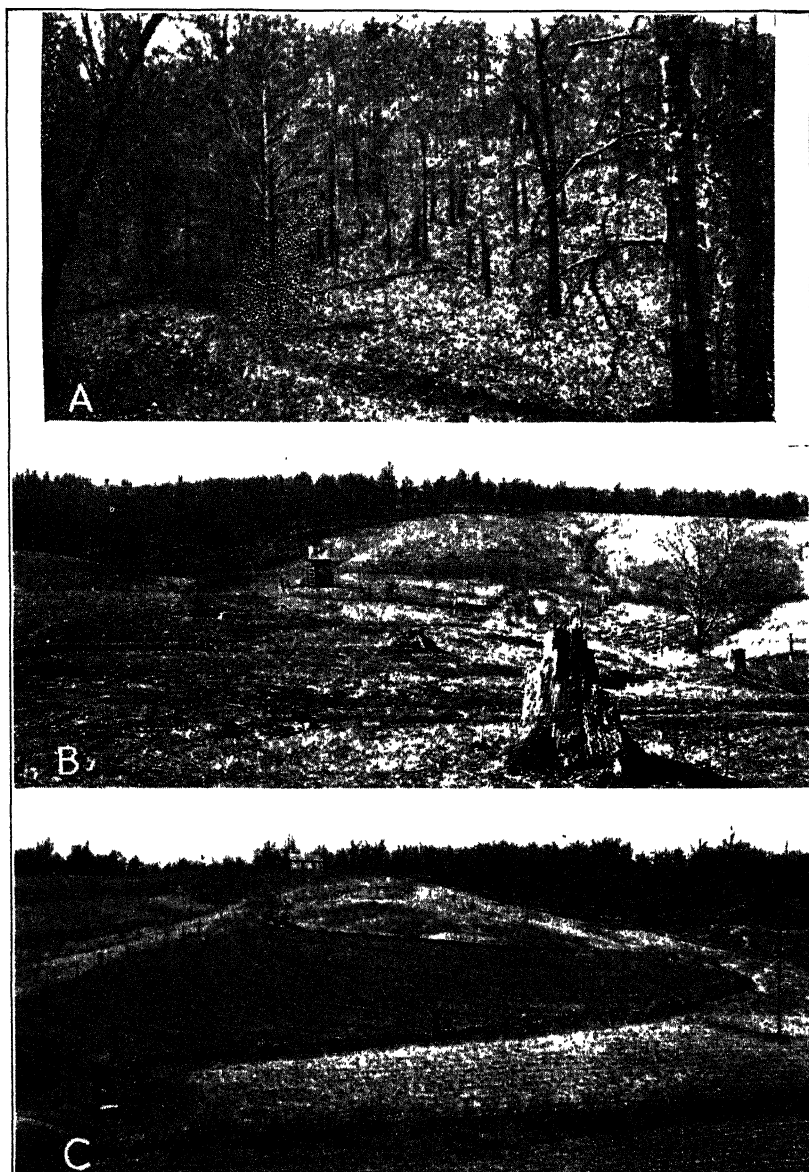
FIG. 8.—A, timbered hillside on watershed No. 2 (note the covering of leaves on the ground); B, hillside on lower portion of watershed No. 3, taken from above rain-gauge station (note the gullies, contour plowing to check erosion, and timbered area on the left of watershed No. 2; C, hillside showing location of rain gauge. This area is part of the lower portion of watershed No. 3 (note the hillside ditches that have developed into deep gullies)

drainage channels of these watersheds unite to form the main channel of watershed No. 3, a short distance above the gauging station. (See fig. 1.) Watershed No. 1 already has been described. Watershed No. 2 contains 20.6 acres, of which 38.8 per cent is timbered. A typical view of a timbered hillside on watershed No. 2 is shown in Figure 8, A. Note the covering of leaves on the ground. Except for a few gullies, the cleared portion of this watershed is comparatively free from erosion. The hilly portion of that part of watershed No. 3 below the junction of the channels of watersheds Nos. 1 and 2 is quite badly gullied and subject to rapid surface washing. (See fig. 8, B and C.) The gullies and the hillside ditches promote the rapid drainage of this portion of the watershed. A view of the lowlands between the hillsides at the lower end of watershed No. 3 is shown in Figure 9, A. A good crop of corn was harvested on this land in 1918. The fall of the channel at the gauging station is 2.2 feet per hundred. The distance from the farthest point on the watershed to the gauging station is 2,152 feet, and the average fall for this distance is 3.99 feet per 100 feet. The maximum fall on the hillsides of the watershed is 64 feet per 100 feet. In Figure 7 is shown a cross section of the channel at the gauging station, with stages for the rains that occurred on April 19 and July 18.

Table 3 gives a summary of the rainfall and run-off measurements for the watershed. The time of concentration for this watershed was taken to be 10 minutes. The maximum rate of run-off for the nine rains listed in the table was 80 cubic feet per second, or 1.61 inches per hour. This was due to a rainfall rate of 3 inches per hour. The rates of rainfall on both April 28 and May 7 were greater but the rates of run-off were less, probably due to the fact that the high rates on those dates occurred near the beginning of the storm, while the maximum rate on July 18 occurred after 1.41 inches of rain had fallen. Referring to Table 3, it is seen that the run-off coefficients for this watershed range from 0.30 to 0.54—practically the same variation as for watershed No. 1—and that the rates of both rainfall and run-off are considerably smaller than for watershed No. 1, as would be expected for the larger area with time of concentration twice that of the smaller watershed.

<center>WATERSHED NO. 4</center>

Watershed No. 4 is fan-shaped and contains 15.7 acres, 38.9 per cent of which is covered with timber. Figure 9, B, is a view of a portion of the watershed. That part of the watershed south of the large timbered area (see fig. 1) is eroded badly. Deep gullies and hillside ditches render it practically impossible to cultivate this land; hence it is used for pasture. Over a large portion of the cleared land on the remainder of the watershed erosion is not so active, and most of this land was in cotton during the period of these investigations. The fall of the channel at the gauging station is 3.5 feet per 100 feet. The distance from the farthest point on the watershed to the gauging station is 1,418 feet, the average fall for this distance being 5.44 feet per 100 feet. The maximum fall on the hillsides of the watershed is 32 feet per 100 feet. Figure 7 shows a cross section of the channel at the gauging station.

Table 4 is a summary of the rainfall and run-off measurements. The time of concentration for this watershed was taken to be seven
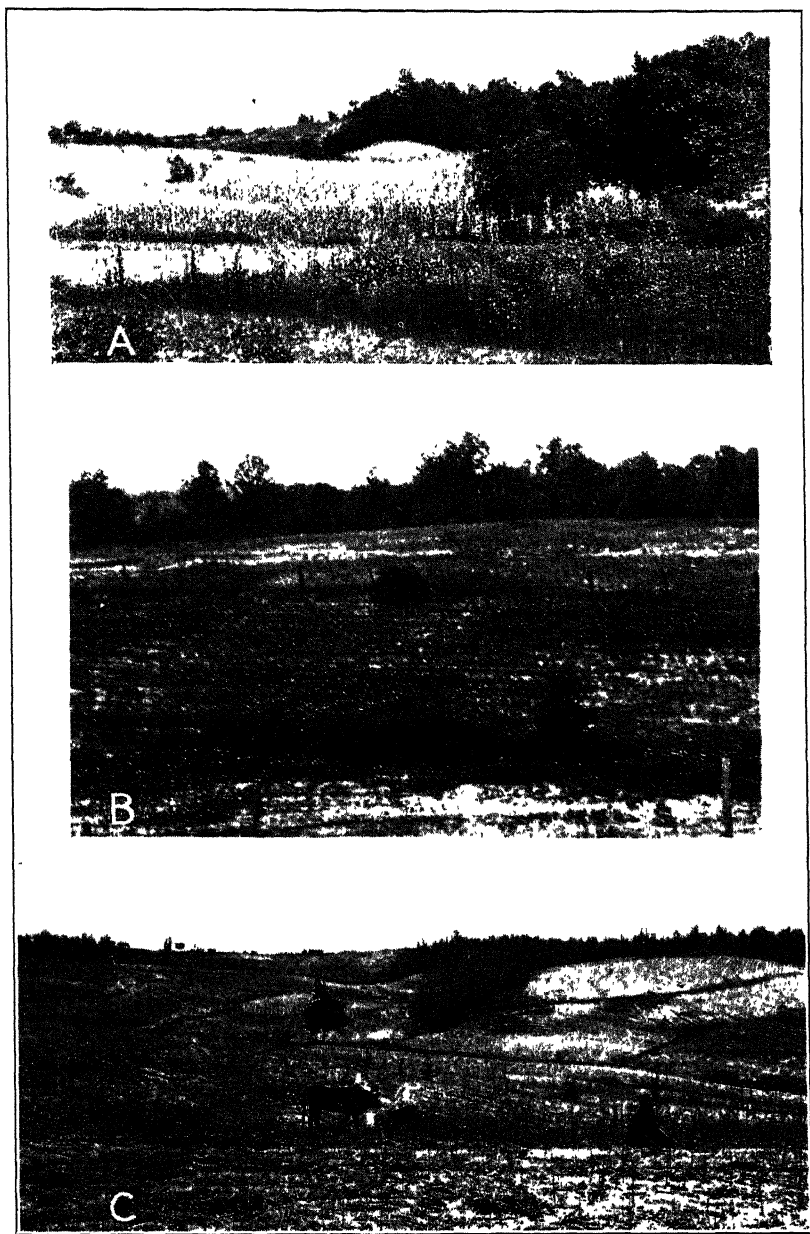
FIG. 9.—A, corn growing on comparatively low land along lower portion of channel of watershed No. 3; B, a portion of watershed No. 4 taken from the rain-gauge station; C, a portion of watershed No. 5 taken from near gauging station No. 6 and looking toward gauging station No. 3

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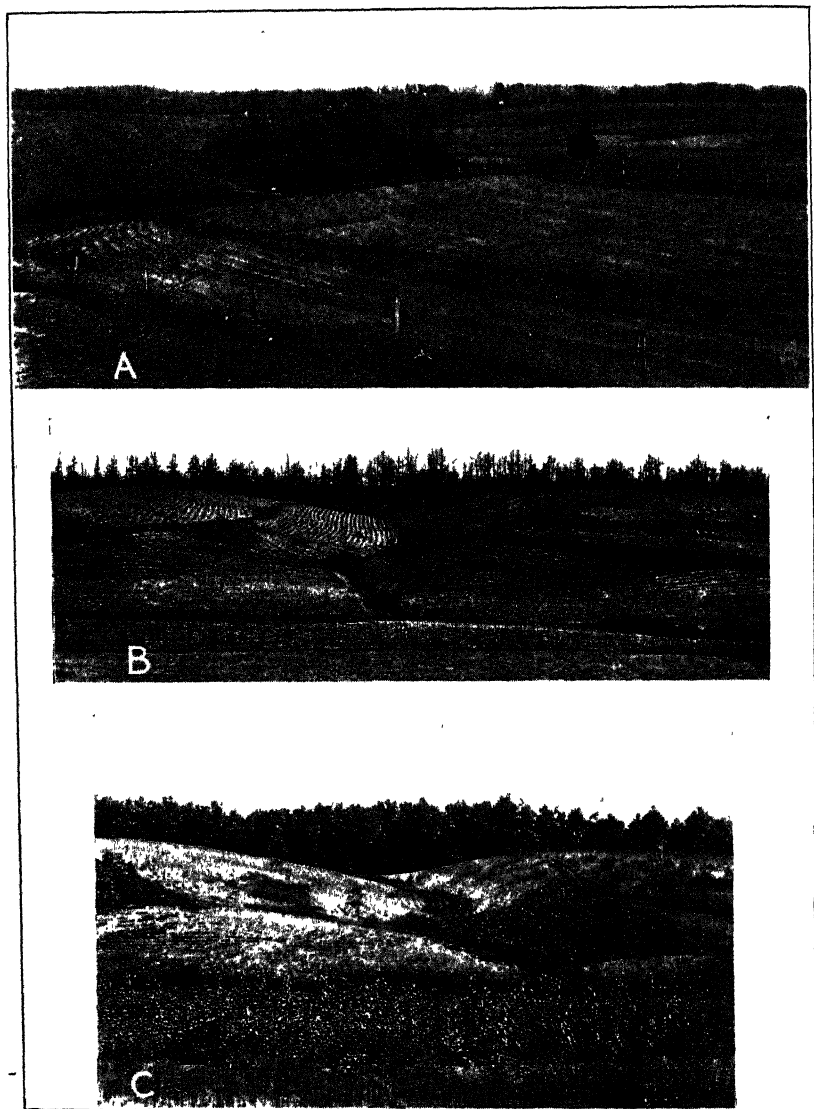
Fig. 10.—A, a portion of watershed No. 5 from rain-gauge station, looking toward gauging station No. 3; B, watershed No. 7, taken in March, 1917; C, watershed No. 7, taken in July, 1917. (Note growth of vegetation)

minutes, which is a little longer than that for watershed No. 1, due
no doubt to the greater percentage of timber on watershed No. 4.
The run-off coefficients vary from 0.22 to 0.46, and are decidedly
lower than the coefficients obtained for watershed No. 1, which fact
also is attributed to the greater percentage of timber on the water-
shed.

<div align="center">WATERSHED NO. 5</div>

Watershed No. 5 contains 112 acres, 23.9 per cent of which is
covered with timber. It includes all of the other watersheds that
have been described and in addition watersheds Nos. 6 and 7 to be dis-
cussed later. From gauging stations Nos. 3 and 4 to gauging station
No. 5 (see fig. 1), there is a comparatively wide and flat area of land
which constitutes the most fertile and productive part of the water-
shed. It has been built up by deposits of soil washed from the hilly
parts of the watershed during overflows of the main channel. Good
yields of corn, cotton, and hay are obtained from this portion of the
watershed. Figures 9, C, and 10, A, give views of portions of watershed
No. 5.

The distance from the farthest point on the watershed to the
gauging station by channel is 3,933 feet and the average fall for this
distance is 2.74 feet per 100 feet. The fall of the channel at the
gauging station is 1 foot per 100 feet and the maximum fall on the
hillsides of the watershed is 64 feet per 100 feet. In Figure 7 is shown
a cross section of the channel at the gauging station, upon which are
indicated the stages of water in the channel for the most intense
rains that occurred. During the heaviest rate of run-off—which
occurred on April 19—it is seen that one of the banks of the channel
was overflowed.

Table 5 is a summary of the rainfall and run-off measurements.
The time of concentration for this watershed was taken to be 17
minutes for all of the rains except that of April 19, for which rain it
was taken as 12 minutes, since the average velocity in the channel
was greater owing to more rapid filling. The maximum rate of run-
off occurred on April 19 and was 333.5 cubic feet per second, or 2.95
inches per hour. The rain of that date fell at the average rate of
3.5 inches per hour for 12 minutes and at the rate of 4.8 inches per
hour for 5 minutes of the 12-minute period. (See fig. 3, C.) The
run-off coefficient for this rain is 0.85, almost twice as large as for
any of the other rains given in the table, which seems to show that
the run-off coefficient is considerably higher for excessive rates than
for normal rates of precipitation. The next greatest rate of run-off
occurred on July 18 and was 124.5 cubic feet per second, or 1.10
inches per hour. The coefficient of run-off for this rain is 0.41.
Except only for the rain of April 19, the coefficient of run-off ranged
from 0.27 to 0.48.

Computations were made for this watershed to determine, for each
rain, the percentage of the total rainfall that ran off. In Figures 11
to 14 are plotted the rates of rainfall in inches per hour for five-
minute periods, as well as curves showing the rates of run-off, in
inches per hour, due to that rainfall. A common scale of ordinates
and abscissas was employed. The area under the run-off curve was
reduced to total inches of run-off and this run-off was computed in
percentage of the total rainfall. For the heavy rain that occurred

on April 19 this percentage is 85.2, or about the same as the run-off coefficient for that date. However, attention is called to the fact that the run-off coefficient and the percentage of rainfall that runs off are not necessarily the same. The percentage running off during the time of concentration may be greater or less than the percentage running off for the entire storm. The other percentages (see Table 5)
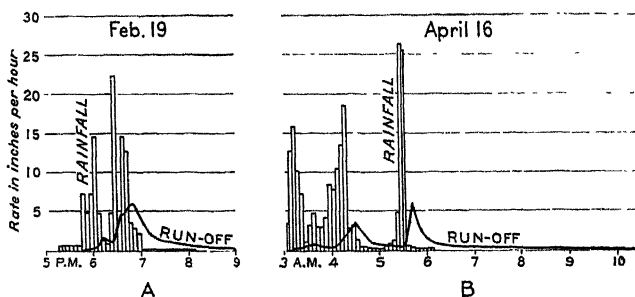
FIG. 11.—Comparative rates of rainfall and run-off, watershed No. 5, February 19 and April 16

range from 29.0 to 51.5. No attempt was made to determine the rates of run-off at the other stations for the rain of April 19, since the stages in the channels were so much higher than for any for which gaugings had been made that only a very rough estimate could be made of the discharges of the channels. It is believed, however, that the results obtained for watershed No. 5, which includes all of the other watersheds, represent in a general way conditions of run-off
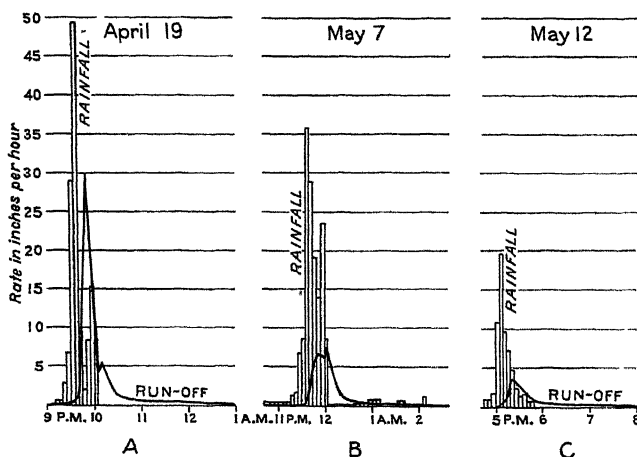
FIG. 12.—Comparative rates of rainfall and run-off, watershed No. 5, April 19, May 7, and May 12

on the other watersheds for this rain. An idea can be obtained from Figures 11 to 14 as to the time relation existing between the maximum rates of rainfall and the maximum rates of run-off. The areas under the rainfall and run-off curves are proportional to the total rainfall and run-off, respectively, so that the relation between the total rainfall and the total run-off for any of the rains can be approximately determined from an inspection of the curves.

WATERSHED NO. 6

This watershed contains 1¼ acres and is the smallest of the several watersheds studied. All of it is cleared and has in time past been in cultivation. However, it has become so badly eroded and barren of soil that it has practically been abandoned to the growth of weeds. The watershed is fan-shaped and the numerous gullies converge
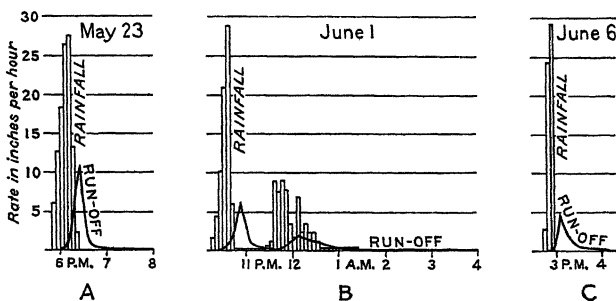
FIG. 13.—Comparative rates of rainfall and run-off, watershed No. 5, May 23, June 1, and June 6

toward the outlet—conditions favorable to a high rate of run-off. The distance from the farthest point on the watershed to the gauging station is 359 feet and the average fall for this distance is 9.78 feet per 100 feet. The maximum fall on the watershed is 33 feet per 100 feet. The time of concentration was judged to be about 1½ minutes, but it was impossible to determine the time accurately from the records of the water-stage recorder.

Table 6 is a summary of the rainfall and run-off measurements. The maximum rate of run-off occurred on April 28 and was 3.88
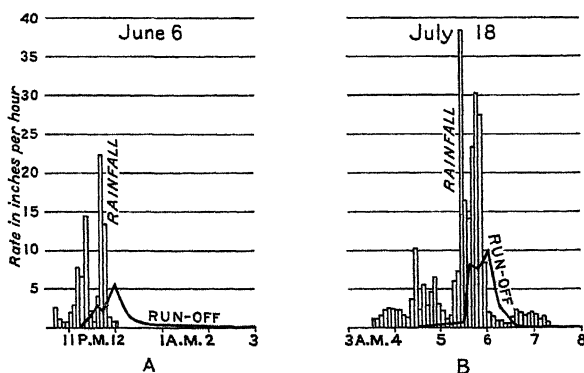
FIG. 14.—Comparative rates of rainfall and run-off, watershed No. 5, June 6, and July 18

cubic feet per second, or 3.08 inches per hour. The maximum average rate of rainfall for this day was 5.2 inches per hour. The run-off coefficients for the 10 rains given in the table range from 0.26 to 0.59.

Measurements of the discharge for this watershed were made with a weir box, as has been described. A catch basin, consisting of the enlargement of the ditch above the weir box was provided to remove

the sand and silt from the run-off water. For the heaviest rains, however, this basin usually was nearly filled and sand was deposited in the weir box, which rendered the weir measurements somewhat inaccurate.

WATERSHED NO. 7

Watershed No. 7 contains 2.79 acres, of which 55½ per cent is covered with timber. Figure 10, B and C, gives two views of this watershed, one taken in March and the other in July. The timber lies on the upper part of the watershed. The lower part, which is cleared, is trough-shaped. Hillside ditches on both of the side slopes intercept the water from the upper portions of the slopes. These slopes are somewhat eroded, as may be seen by a close inspection of Figure 10, B and C, but not as much as are the slopes on watershed No. 6. The cleared portion of this watershed was not cultivated during the period of these investigations. The distance from the farthest point on this watershed to the gaging station is 672 feet, and the average fall for this distance is 8.53 feet per 100 feet. The maximum fall on the slopes of the watershed is 39 feet per 100 feet. The time of concentration was estimated to be three minutes, or twice as long as that of watershed No. 6.

A summary of the rainfall and run-off measurements for this watershed is given in Table 7. The maximum rate of run-off occurred on April 28 and was 5.03 cubic feet per second, or 1.79 inches per hour. This rate was due to a rainfall at the rate of 5.2 inches per hour. The run-off coefficient for this rain is 0.34 as compared with a coefficient of 0.59 for watershed No. 6 for the same rain. The run-off coefficients for this watershed vary from 0.18 to 0.34, while those for watershed No. 6 range from 0.26 to 0.59. This great difference in the coefficients is no doubt due principally to the timbered area on watershed No. 7 and to the fact that the cleared area on No. 7 was less eroded and cut up with gullies than was watershed No. 6.

Measurements of the discharge from this area were made with a weir box similar to the one used for watershed No. 6 and the results were subject to the same sort of inaccuracies, though in less degree, as were mentioned for watershed No. 6.

EFFECT OF TIMBER UPON RATE OF RUN-OFF

The effect of timber upon the rate of run-off is shown by a comparison of the results obtained for watersheds Nos. 1 and 4, where the timbered areas were 14 and 38.9 per cent of the respective watersheds. In Table 8 it is seen that the run-off coefficients for watershed No. 1 for all rains except that of July 18, range from 0.33 to 0.49, and for watershed No. 4, from 0.22 to 0.35. These values show quite conclusively that timber has a decided influence in reducing the rate of run-off from a watershed. However, the results obtained for the rain of July 18—for which run-off coefficients of 0.51 and 0.46 for watersheds Nos. 1 and 4, respectively, were obtained—tend to show that the effect of timber in reducing run-off is slight when the maximum rate of run-off occurs after considerable rain already has fallen. This is to be explained by the fact that interception and percolation on timbered areas are much greater at the beginning of a rain than later, so that an increasingly greater proportion of the rainfall runs off as the rain continues. The falling rain is inter-

cepted by the trees, and the cover of leaves on the ground, until saturated, absorbs a large portion of the rainfall. On April 28 the average rates of rainfall were greater than on July 18, yet the rates and coefficients of run-off were smaller, being 0.41 and 0.25 as compared with 0.51 and 0.46 for the rains of April 28 and July 18, respectively. However, the rain that fell prior to the time of concentration on April 28 was 0.09 inch for both watersheds, while on July 18 it was 0.76 inch for watershed No. 1 and 0.71 inch for watershed No. 4.

TABLE 8.—*Effect of timber on run-off coefficients for watersheds Nos. 1 and 4; watershed No. 1, 14.0 per cent in timber; watershed No. 4, 38.9 per cent in timber*

Date of rain, 1918	Average rate of rainfall during time of concentration		Coefficient of run-off (ratio of maximum rate of run-off to average rate of rainfall)		Rainfall prior to period taken as time of concentration	
	Water-shed No. 1	Water-shed No. 4	Water-shed No. 1	Water-shed No. 4	Water-shed No. 1	Water-shed No. 4
	Inches per hour	*Inches per hour*			*Inches*	*Inches*
Feb. 19	2.40	1.89	0.40	0.22	0.39	0.37
Apr. 16	3.84	2.92	.33	.29	1.21	1.19
Apr. 28	4.44	3.77	.41	.25	.09	.09
May 7	3.60	3.51	.38	.28	.18	.18
May 12	1.92	1.89	.41	.30	.10	.10
May 23	3.12	2.83	.49	.35	.38	.38
June 1	3.24	3.00	.36	.28	.46	.38
June 6, 11 p. m	2.28	2.14	.42	.28	.72	.69
July 18	4.20	3.51	.51	.46	.76	.71

SOME FACTORS INFLUENCING THE RAINFALL AND RUN-OFF RELATION

A comparative study of the rates of rainfall and run-off for the different storms given in Tables 2 to 7 shows what apparently are unaccountable variations in the run-off coefficients. There are many interdependent factors entering into the relation between rainfall and run-off and it is practically impossible to evaluate all of them accurately. For instance, the effect of previous rains upon the capacity of the ground to absorb water from subsequent rains depends upon the nature and amount of previous rainfall, the interval of time between rains, and the amount of water lost through transpiration and evaporation during this interval. If the maximum rate of rainfall occurs at the beginning of the rain, before the surface of the ground has been thoroughly wetted, the percentage of run-off is less than when the maximum rate occurs some time after the beginning of the rain. A greater percentage of run-off also occurs for heavy than for light rains, an illustration of which is the rain of April 19 on watershed No. 5, when the percentage of run-off was 85.2 as compared with the next highest percentage of 51.5 for the comparatively light rain on June 6 on this watershed. (See Table 5.) For the storm of April 19 the time of concentration was less than for the other storms given in the table, because the ditches filled more rapidly and the average velocity of flow was greater. Nonuniformity in the rates of rainfall during the time of concentration is responsible for considerable variation in the run-off coefficient for the same water_

shed. An idea as to the degree of uniformity of the rates of precipitation during the time of concentration can be formed from a study of the rainfall charts shown in Figures 3 and 4.

THE RATIONAL METHOD OF COMPUTING RUN-OFF

In the rational method of computing run-off the various factors influencing run-off are provided for in the formula $Q = C I A$. C, the coefficient of run-off, is the composite effect of all factors influencing run-off which have been mentioned. I, the rate of rainfall to be provided for, depends upon the intensity for different durations of rainfall for the particular locality, and the duration to be used for any particular watershed is equal to the time of concentration of that watershed. Thus the time of concentration takes care of such influencing factors as the shape and slopes of the watershed and the arrangement and character of the drainage channels. To a certain extent, also, it takes account of the vegetation on the watershed, since the distance traveled and the velocity of the water depend partly upon these factors.

PROBABLE FREQUENCY OF RAINFALL INTENSITIES

In the design of drainage channels for small watersheds it is important to know not only the rates of precipitation to be expected for short periods of time but also the probable frequency of the occurrence of these rates. For most improvements it would be impracticable to make provision for the greatest rates of rainfall ever recorded. A matter for the engineer to decide, then, relates to the frequency with which it is permissible that the improvement be over-taxed—that is, whether he shall plan to provide for such intense rates of rainfall as occur but once in, say, 50 years, or whether he shall adopt a frequency of 25 years, or 10 years, or 1 year. In reaching his decision the engineer must, of course, take account of all of the interests involved.

The probable frequency of different rates of precipitation for storms of various durations is shown by the curves in Figure 15. These curves were plotted from precipitation data in Table 15 of Meyer's Elements of Hydrology,[5] which data were compiled from the United States Weather Bureau records taken at the following 19 stations, for the period 1896 to 1914: Boston, Albany, Pittsburgh, Elkins, Asheville, Knoxville, Memphis, Cairo, Indianapolis, Cincinnati, Cleveland, Detroit, Grand Haven, Chicago, Madison, St. Paul, Moorhead, Yankton, Dodge City. These stations showed similar rates of precipitation and were grouped together for the purpose of indicating the probable frequency of rains during a period of time longer than the periods for which the records were available at each individual station. From these curves the engineer can readily determine what rate of rainfall intensity should be adopted as the basis of his improvement, having first decided with what frequency he would be justified in permitting the capacity of the proposed improvement to be exceeded.

[5] MEYER, A. F., THE ELEMENTS OF HYDROLOGY. 487 p , illus. New York and London. 1917.

APPLICATION OF RESULTS

The results of these investigations are applicable to the design of open-ditch drainage systems, graded-terrace systems, small diversion ditches, storm-water sewers draining in part agricultural areas, tile drains with surface inlets, road culverts, and other improvements required to care for the run-off from small watersheds. It should be understood, however, that for the direct application of these results to specific projects, it is necessary that the nature and characteristics of the watersheds involved be essentially similar to those of the watersheds herein described. To cite an extreme case, it would be incorrect to assume for flat watersheds run-off coefficients identical with those obtained in these investigations on hilly land.
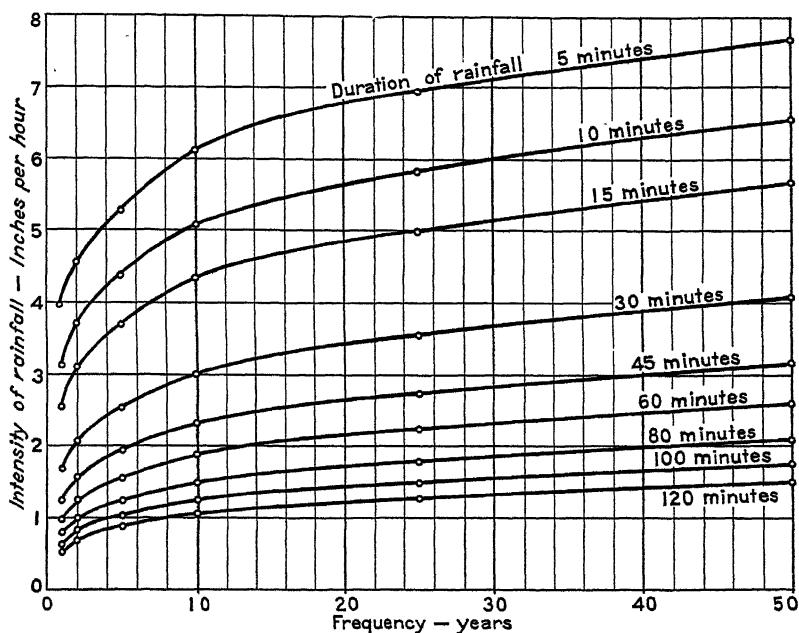
Fig. 15.—Curves showing relation between frequency and intensity of rainfall for storms of various duration

In determining the probable rate of run-off from any particular watershed the rational method should be employed. Values of C, I, and A should be determined for substitution in the equation $Q = C I A$. A, the watershed in acres, should be determined from a map if available, or from a survey of the watershed. C, the run-off coefficient, should be selected from the experimental values for C given in Tables 2 to 7, such revisions being made as seem necessary to account for differences in characteristics. I, the rate of rainfall in cubic feet per second per acre—for practical purposes this is the rate of rainfall in inches per hour—depends upon the time of concentration for the watershed, and upon the frequency period decided upon. The time of concentration can be determined approximately by measuring the distance and estimating the average velocity from the most remote point to the outlet of the watershed, by channel.

Where the proposed improvement consists simply of a drainage ditch, occasional overflows of which would do little harm, it may be sufficient to make provision for such heavy rains as occur but once or twice a year. In the case of a road culvert through an expensive hard-road embankment it may be advisable to provide for such rains as have a frequency of once in 20 or even 50 years.

After determining the time of concentration and deciding upon the probable frequency of the rain to be provided for, the rate of rainfall that applies can be determined from the curves in Figure 15. For instance, if the time of concentration is 10 minutes and provision is to be made for the greatest rain that occurs once in four years, then the curve "10 minutes" gives that rate of rainfall as about 4.2 inches per hour.

As an example, let it be required to estimate the rate of run-off to be provided for in a channel in Tennessee draining a watershed area of 28 acres with characteristics similar to those described for watershed No. 1. The distance by channel is 1,800 feet from the most remote point on the watershed to the outlet and it is desired to provide capacity in the channel for such a maximum rate of rainfall as occurs once in two years.

The time of concentration for the watershed may be assumed to be the same as that given in Table 1 for watershed No. 1, namely, 5 minutes. Thus the velocity of flow from the most remote point of watershed No. 1 to the outlet is $\frac{1,220}{5} = 244$ feet per minute. Assuming the velocity to be practically the same for the watershed of the proposed channel, then the time of concentration would be $\frac{1,800}{244} = 7.38$ minutes, or, say, $7\frac{1}{2}$ minutes. Referring to Figure 15 it is seen from the curves that the maximum rate of rainfall occurring once in two years for a duration of $7\frac{1}{2}$ minutes is about 4.1 inches per hour. This value is found halfway between the 5 and 10 minute curves along the two-year ordinate. Now, referring to the values of C obtained for watershed No. 1, as given in Table 2, it is seen that these values range from 0.33 to 0.51, and for rains with rates of 4.44 and 4.20 inches per hour they are 0.41 and 0.51, respectively. Judging from these data, a value of 0.50 would be satisfactory for this watershed. For the equation $Q = C I A$ we now have $C = 0.50$, $I = 4.1$, $A = 28$. Making the substitutions we get $Q = 0.50 \times 4.1 \times 28 = 57.4$ cubic feet per second as the required capacity of the proposed channel.

FURTHER INVESTIGATION DESIRABLE

As far as the writer knows, these investigations are the first of the kind that have been made to determine rates of run-off in open channels from purely agricultural areas where self-recording instruments were employed. It is fully recognized that the results secured cover merely a small portion of the whole field for investigation, but it is believed that they will prove of considerable value in that small portion and of some value as a dependable guide in the unexplored portion of the field. Excluding mountainous areas, these experiments cover one of the extremes in the topography of watersheds, the opposite extreme being watersheds with very little fall to carry off the water. Between these two extremes lie watersheds

with topography commonly described as slightly rolling, rolling, and moderately hilly. Pending complete investigation of the entire field, fairly accurate estimates can be made for the intermediate types of topography after complete investigations have been made for watersheds of the two extreme types. Of course, many factors besides topography affect the value of the run-off coefficient; all of these should be carefully considered in investigations for comparative purposes in order that the measure of their effects may be more accurately determined as the field covered by the investigation expands.

STRAINS OF KERNEL SMUTS OF SORGHUM, SPHACELOTHECA SORGHI AND S. CRUENTA[1]

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INTRODUCTION

In comprehensive varietal experiments with sorghums in the years 1915 to 1921, inclusive, Reed and Melchers[3] found that milo, feterita, and hegari remained almost free from covered kernel smut, *Sphacelotheca sorghi* (Link) Clinton. In 1923 kernel smut was reported as occurring in milo in northwestern Kansas, northeastern New Mexico, and northwestern Texas.[4] Smutted heads of plants identified as milo were obtained from Colby, Kans., Clovis, N. Mex., and Dalhart and Plainview, Tex. These heads in most cases were only partially smutted.

Plans for investigating the cause of this outbreak of smut in milo were made independently by the Office of Cereal Crops and Diseases and the Kansas Agricultural Experiment Station. Preliminary experiments by the Office of Cereal Crops and Diseases at Arlington Experiment Farm, Rosslyn, Va., in 1923–24 showed that the smut spores from milo produced fairly high percentages of smut in milo. In the autumn of 1924 a limited survey of the infested region in the Southwest revealed kernel smut in fields of both hegari and milo. It was apparent that sorghum mixtures or hybridization in which susceptible parents were involved was not the explanation for the occurrence of smut in commercial sowings of milo and hegari. Following this survey, cooperative investigations were planned by the Offices of Cereal Crops and Diseases and of Dry-Land Agriculture of the Bureau of Plant Industry, United States Department of Agriculture, and the Kansas Agricultural Experiment Station, for the purpose of comparing the smut from milo with the ordinary covered kernel smut of sorghum. A large number of varieties and selections of the various groups of sorghums have been grown in order to compare the pathogenicity of the two smuts and to determine the specific reaction of the various sorghums to the smut from milo. Study was made also of the identity of the milo smut fungus. It is the purpose of this paper to present the results of these investigations.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 9
May 1, 1927
Key No. G-573

(825)

<div align="center">PRELIMINARY EXPERIMENTS</div>

Seed of milo from several sources, including seeds from partially smutted heads obtained from southwestern fields, was inoculated by dusting thoroughly with spores of the smut from milo, and sown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., November 15, 1923. Of the 167 plants 5 were smutted. Three of these smutted plants grew from seed obtained from partially smutted heads. A similar experiment was conducted at Arlington Experiment Farm in 1924. Seed of milo from several sources was inoculated with smut from milo collected in different localities and sown in the spring of 1924. The plants grown from this inoculated seed showed high percentages of smutted heads. The results of this experiment are given in Table 1.

TABLE 1.—*Infection of different lots of Dwarf and Standard Yellow milo by kernel smut, Sphacelotheca sorghi, collected from individual heads of milo, kept separately, when grown at Arlington Experiment Farm, Rosslyn, Va., in 1924*

Variety of milo	Source in 1923, of—		Total number of heads	Number of smutted heads	Percentage of smutted heads
	Seed	Smut for inoculation			
Standard Yellow	Clovis, N. Mex. (smutted head)	Not inoculated	219	1	0.5
Do	____do	Same head	116	43	37.1
Dwarf Yellow	Colby, Kans. (smutted crop)	Not inoculated	219	5	2.3
Do	____do	Clovis	226	56	24.8
Do	Dalhart, Tex	Not inoculated	269	0	0
Do	____do	____do	299	0	0
Do	____do	____do	232	3	1.3
Do	Dalhart, Tex. (smutted head)	____do	241	0	0
Do	Dalhart, Tex	Dalhart	359	60	16.7
Do	____do	____do	303	35	11.6
Do	____do	Plainview	346	55	15.9
Do	Dalhart, Tex. (smutted head)	Same head	154	22	14.3
Do	____do	____do	162	28	17.3
Do	____do	Dalhart	290	82	28.3
Do	Plainview, Tex	Not inoculated	283	0	0
Do	____do	Plainview	305	6	2.0
Do	____do	____do	257	26	10.1
Do	Plainview, Tex. (smutted head)	Not inoculated	325	0	0
Do	____do	Same head	289	102	35.3
Do	____do	____do	499	46	9.2
Do	Clovis, N. Mex. (smutted head)	Plainview	420	51	12.1

The data in Table 1 show that Standard Yellow and Dwarf Yellow milos are strongly susceptible to the kernel smut from milo when grown under the conditions prevailing at Arlington Experiment Farm.

In the winter of 1924–25 another experiment was conducted in the greenhouse at Arlington Experiment Farm. Seed of several sorghum varieties, including Dwarf Sumac, White milo, Dwarf Yellow milo, Standard Yellow milo, feterita, a broomcorn, and three selections from a cross (Red Amber × feterita) was inoculated, a part with smut from milo, a part with smut from hegari, and a part with the ordinary covered kernel smut from Kansas Orange grown at Manhattan, Kans. The seed was sown January 6, 1925. Feterita and two of the Red Amber × feterita hybrids remained smut free regardless of the source of inoculum. The third selection of Red Amber × feterita showed very low percentages of smutted heads on plants grown from seed inoculated with smut from hegari, broomcorn, and milo, but no smut occurred in the plants grown from seed inoculated with the ordinary kernel smut. The broomcorn smut was collected at Texline, Tex., and may probably be the strain occurring in milo. All smut lots produced fairly high percentages (5.9 to 40 per cent) of smutted heads in broomcorn.

Only smut from hegari and milo produced smut in milo. Of the 15 heads of White milo produced from seed inoculated with smut from hegari, 3 were smutted, while only 3 heads out of 34 were smutted when the seed was inoculated with smut from milo. One head in 11 of Dwarf Yellow milo was smutted when spores from hegari were used and one in 27 when smut from milo was used. Two heads in 18 of Standard Yellow milo were smutted when spores from hegari were used. All other plants were smut free. These records seemed to indicate that the smut from hegari is more pathogenic on milo than is the smut from milo itself, but this conclusion would have to be verified by further work. The smut from hegari produced no smut in Dwarf Sumac, however, while all other smut lots produced from 14.3 to 63.6 per cent of smut in this variety.

VARIETAL EXPERIMENTS IN 1925 AND 1926

In the spring of 1925 sowings were made in the cooperative varietal experiment. The object of this experiment was to grow representative varieties of the important groups of sorghums so as to note their reaction to infection by the smut from milo as compared with their reaction to the covered kernel smut commonly occurring in sorghum. In addition to the varieties, several hybrids which had been bred for smut resistance were included.

Smut for these experiments was collected at Dalhart, Tex., from milo and hegari and shipped to Washington, D. C., where the smutted heads were ground in a meat chopper and the spores sieved out and used for inoculating the seed. The ordinary covered kernel smut was collected from susceptible sorghums at Manhattan, Kans., where smut on milo does not occur naturally. This spore material was carefully packed and sent to Washington, where the seed was inoculated.

Seed of the various sorghums, furnished by the departments of agronomy and botany and plant pathology of the Kansas Agricultural Experiment Station from smut-free plots, was sent to Washington, where each lot was divided into six equal parts. Three parts of each lot were smutted with milo smut and three with ordinary covered kernel smut. A set of the seed inoculated with each kind of smut was sown at each of three different stations, located at Rosslyn, Va., Dalhart, Tex., and Manhattan, Kans. In order to be positive that it was free from smut spores at the time of inoculation, seed used in 1926 was treated by soaking for 30 minutes in a 1:320 solution of formaldehyde, then washed thoroughly in water, and dried before the inoculum was applied. Sufficient spore material was used to make the seed appear dark in color, which is more than naturally occurs in badly infested seed lots.

The seed was sown in rows sufficiently long to produce 100 plants if the stands were reasonably good. In making the records the total number of heads and the number of smutted heads were recorded. In many rows there were fewer than 100 heads, while in others the number exceeded 100. The smut percentages were calculated on the basis of heads smutted.

The data obtained in these experiments are given in Table 2. No attempt was made to strike averages, as only two years' data are available and the higher percentage of infection seems more nearly to represent the potential susceptibility of a variety than does the average.

The data in Table 2 show that the sorghums previously known to be susceptible to covered kernel smut are about equally susceptible to both the strains of smut used, namely, the special strain from milo and hegari and the ordinary strain of covered kernel smut. The sorgos, kafirs, kaferitas, broomcorns, kaoliangs, and the miscellaneous varieties, with the exception of White Yolo, fall into this division.

TABLE 2.—*Reaction of varieties and hybrids of sorghum to infection by a strain of kernel smut occurring on milo, and by the ordinary covered kernel smut, Sphacelotheca sorghi, when grown at Rosslyn, Va., Dalhart, Tex., and Manhattan, Kans., in 1925 and 1926*

Group and variety, strain, or selection	Kansas plot No. or nursery row No. (1924), from which seed was taken	Percentages of infection on sorghum grown from seed inoculated with—											
		Ordinary covered kernel smut						Smut strain from milo					
		Rosslyn, Va.		Dalhart, Tex.		Manhattan, Kans.		Rosslyn, Va.		Dalhart, Tex.		Manhattan, Kans.	
		1925	1926	1925	1926	1925	1926	1925	1926	1925	1926	1925	1926
Sorgo:													
Dwarf Sumac	Plot 4	23.5	19.2	50.0	10.1	26.2	43.1	9.0	11.9	40.6	15.8	14.9	25.6
Fielding Sumac	Plot 3	23.3	28.5	60.8	83.1	48.6	55.6	19.8	7.1	9.5	24.1	27.4	44.3
Red Amber	Plot 31	9.9	15.6	18.4	10.2	10.3	7.0	7.1	12.0	11.0	6.2	24.4	15.0
White African	Plot 10	28.3	23.2	39.7	40.7	45.1	32.0	26.2	8.7	56.4	21.6	42.7	43.6
Kansas Orange (head selection)	Field	2.2	20.0	40.7	7.9	18.8	15.5	1.0	1.6	27.0	11.3	6.3	5.2
Lasley	Row 314	0	11.7	14.4	8.5	26.2	50.0	0.9	8.1	5.7	16.4	6.9	44.9
Kansas Orange, black glumes	Plot 1	3.6	19.1	45.9	0	33.0	17.4	4.1	10.0	26.5	0	10.8	14.0
Leoti Red, F. C. I. 6610		10.3	14.8	11.8	22.8	11.5	23.6	24.0	26.5	19.9	24.1	19.6	10.9
Red Amber × feterita, F7 hybrids:													
Selfed selection	Row 12	0	0	0	0	0	0	0	0	7.4	1.4	0	14.7
Head selection	Row 18	0	0	0	0	0	0	0	0	25.5	1.0	15.6	3.0
Selfed selection	___do	0	0	0	0	0	0	0	0	28.3	2.9	12.1	5.4
Do	Row 13	0	0	0	1.0	0	0	0	0	36.0	8.4	0	10.2
Do	Row 12	0	1.1	5.6	1.4	0	1.1	0	0	0	0	0	0.8
Do	Row 73	0	0	0	0	0	0	0	0	0	0	0	0
Do	Row 69	0	0	0	0	0	0.5	0	0.8	5.1	6.7	4.1	6.7
Do	Row 42	0	0	0	0	0	0.6	0	0	4.3	0	1.4	0.7
Do	Row 40	0	0	0	1.3	2.5	0	0	0	2.0	0	0	0.5
Do	Row 39	1.6	0	0	1.0	24.1	1.6	0	0	3.6	0	0	0.5
Do	___do	0	1.9	0	1.5	9.4	2.4	0	0	4.8	0.8	0	3.1
Do	Row 25	0	0	0	0	0	0	0	0	0	0	0	0
Head selection	Row 69	0	0	0	0	3.9	1.7	1.8	0	15.6	2.8	0	11.0
Selfed selection	Row 57	0	5.2	19.1	29.2	20.2	16.1	0	0.9	4.2	32.9	0	9.0
Do	Row 56	0	0	0	0	0	0	0	0	0	3.1	0	0
Do	Row 66	0	0	0	8.5	0	0	0	0	10.7	17.7	0.9	6.5
Feterita:													
Spur (selfed selection)	Row 201	0	0	0	0	0	0	0	0	0	0	0	0
Red Leaf (selfed selection)	Row 202	0	0	0	0	0	0		0			0	0
Do	___do		0	0	0.5	0	7.7		0	0	0	0	0
C. I. 182-1		0	0	0	0	0.5	0	0	0	0	0	0	0
Selfed selection	Row 210	0	0	0	0	0	0	0	0	0	0	0	0
Hybrid, F. C. I. 8926		0					4.8		0				0
S. P. I. 51989 (1921 seed)		0							0				
S. P. I. 51989 (1920 seed)		0							0				
S. P. I. 51991 (1920 seed)		0							0				
S. P. I. 51989 (1925 seed)		0					0		0				0
S. P. I. 51991 (1925 seed)		0					1.9		0				0
Kafir:													
Reed	Plot 14	9.2	12.3	34.0	7.1	23.8	41.3	9.3	8.1	49.2	16.3	23.8	20.6
Pink	Plot 20	15.8	22.7	20.3	21.1	26.0	61.6	13.5	20.9	23.7	13.6	18.5	33.3
Wonder (Bower's)	Row 291	4.6	13.3	37.5	6.8	15.7	47.3	4.3	15.1	30.2	20.8	6.1	40.2
Sunrise	Plot 22	6.5	12.1	40.0	12.0	12.1	32.3	6.3	7.0	36.8	19.8	12.6	31.0
Blackhull	Plot 6	9.2	8.4	12.5	12.6	29.4	45.8	5.8	4.1	33.3	24.4	9.7	20.7
Dawn	Plot 23	8.7	12.9	38.5	26.4	35	36.8	6.9	8.0	37.2	16.4	14.7	15.1
Blackhull (head selection)	Field	15.4	9.8	4.3	15.7	21.3	22.6	1.6	6.6	12.7	26.9	5.0	24.6
Red	Plot 19	14.6	8.5	28.6	8.9	46.2	43.9	30.8	5.0	32.8	17.0	21.0	18.4
Pink (head selection)	Field	15.2	12.1	49.0	19.8	37.8	11.6	27.2	10.4	31.9	28.2	4.0	37.6
Wonder (Bower's)	Row 291	7.4	13.2	42.1	14.3	19.2	72.1	6.6	11.8	41.0	10.9	4.5	42.9
Farmer (1923 seed)	Row 401	20.8	10.1	38.9	28.6	27.5	23.8	13.3	2.3	14.3	29.0	25.0	35.7
Dawn (selection Hays Cereal 2421)							22.4						4.4

TABLE 2.—*Reaction of varieties and hybrids of sorghum to infection by a strain of kernel smut occurring on milo, and by the ordinary covered kernel smut, Sphacelotheca sorghi, when grown at Rosslyn, Va., Dalhart, Tex., and Manhattan, Kans., in 1925 and 1926*—Continued

Group and variety, strain, or selection	Kansas plot No. or nursery row No. (1924), from which seed was taken	Percentages of infection on sorghum grown from seed inoculated with—											
		Ordinary covered kernel smut						Smut strain from milo					
		Rosslyn, Va.		Dalhart, Tex.		Manhattan, Kans.		Rosslyn, Va.		Dalhart, Tex.		Manhattan, Kans.	
		1925	1926	1925	1926	1925	1926	1925	1926	1925	1926	1925	1926
Kafir × feterita:													
F. C. I. 8920	Plot 26	7.9	1.4	35.0	1.6	1.6	8.3	18.7	3.2	41.5	7.7	9.2	10.4
F. C. I. 8929	Plot 28	15.2	4.0	40.5	1.3	9.0	1.7	9.6	5.4	30.8	6.1	15.9	15.1
Hays Cereal 2423			0						0	0			0
Kafir-milo hybrids.													
26-3-1-1, (F6 Woodward, Okla. 1924)		0	0	2.1	0	2.2	0	0	1.9	22.0	7.8	3.9	31.0
38-1-2-1, (F6 Woodward, Okla. 1924)			3.8			17.1			5.8			21.0	
Smith's, C. I. 808		7.9		14.1		17.1		6.8		13.3		12.1	
Dwarf Yellow × Pink, Hays Cereal 244							3.2						4.1
Dwarf Yellow × Pink, Hays Cereal 257							0						8.3
Dwarf Yellow × Pink, Hays Cereal 2510							7.9						10.8
Milo × feterita:													
F. C. I. 8916	Plot 25	1.2	0	13.8	0	4.6	0	1.9	0	9.3	0	0	0
Milo:													
Dwarf Yellow, selfed (very juicy, nonsweet).	Row 6 (Bot.[a])	0	0	0	0	0	0	0	0	12.5	6.1	6.8	17.5
Dwarf Yellow, selfed (juicy, nonsweet).	Row 5 (Bot.[a])	0	0	0	0	0	0	0	0	18.1	4.3	0	17.9
Dwarf Yellow, selfed (juicy, nonsweet).	Row 4 (Bot.[a])	0	0	0	0	0	0	0	0	5.4	3.7	0	8.3
Dwarf Yellow, selfed (extremely juicy and sweet).	Row 3 (Bot.[a])	0	0	0	0	0	0	0	1.9	22.8	11.5	0	26.7
Dwarf Yellow, selfed (extremely juicy and sweet).	Row 2 (Bot.[a])	0	0	0	0	0	0	0	2.2	7.1	6.3	0	12.5
Dwarf Yellow, selfed (pithy, nonsweet).	Row 11 (Bot.[a])	0	0	0	0	0	0	4.5	1.2	15.0	13.4	0	2.5
Standard Yellow, C. I. 234		0	0	0	0	0	0	0	0	27.6	5.0	10.1	12.2
Dwarf Yellow, selfed (pithy, sweet).	Row 9 (Bot.[a])	0	0	0	0	0	0	0	5.3	2.5	1.5	0	14.3
Standard White, C. I. 352		0	0	0	0	0	0	3.5	0	20.6	10.9	16.4	2.9
Dwarf Yellow (pithy, sweet).	Row 8 (Bot.[a])	0	0	0	0	0	0	5.9	3.1	10.3	1.3	0	8.9
Dwarf Yellow, C. I. 332		0	0	0	0	0	0	0	0.7	20.3	3.4	12.1	12.1
Dwarf Yellow (pithy, nonsweet).	Row 12 (Bot.[a])	0	0	0	0	0	0	0	2.4	27.0	9.3	2.5	11.9
Do.	Row 10 (Bot.[a])	0	0	0	0	0	0	0	0	10.1	10.1	0	13.7
Dwarf Yellow (pithy, sweet).	Row 7 (Bot.[a])	0	0	0	0	0	3.8	0	1.9	16.7	0	0	9.2
Dwarf White, F. C. I. 8929		0	0	0	0	0	0	2.2	1.0	8.7	12.1	9.8	22.9
Cream	Row 351, 1923	0	0	0	2.3	0	0	4.2	1.7	12.6	2.2	9.5	20.9
Fargo Straightneck, C. I. 809			1.1			9.7		0		18.9			12.1	
Kansas Orange × Dwarf Yellow milo:													
Selfed selection 1, 1925	Row 439					11.7							0
Selfed selection 2, 1925	do					0							4.5
Selfed selection 3, 1925	do					0							5.9
Selfed selection 4, 1925	do					9.0							28.6
Selfed selection 6, 1925	do					4.3							16.7
Selfed selection Remnant, 1924						26.3							50.0
Blackhull × Sourless.													
Selection	Plot 13	15.9	18.7	34.6	21.8	48.4	55.6	20.8	5.8	14.1	27.1	31.3	28.0
Hegari:													
Head selection	Field	0	0	0	0	0	0	2.2	0	17.2	0	0	0
Bulk seed	Plot 18	1.4	0	0	0	0	0	14.1	2.0	46.3	3.1	8.1	0
Broomcorn:													
Standard, C. I. 583		6.0	15.3	11.0	5.3	27.5	5.0	3	15.9	4.9	2.9	3.9	2.5
Acme, C. I. 243		13.2	23.8	18.5	9.4	30.8	17.1	17.0	11.0	16.7	2.5	16.5	15.5
Kaoliang:													
Dwarf Shantung, C. I. 293		3.5	24.5	23.4	1.4	8.9	0	4.9	19.2	21.4	4.0	25.9	4.6
Manchu Brown, C. I. 171		1.1	11.1	2.2	0	2.1	3.7	0.9	18.2	5.6	3.2	10.2	9.4

[a] The abbreviation "(Bot.)" refers to the row number in the nursery of the Department of Botany.

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Group and variety, strain, or selection	Kansas plot No. or nursery row No. (1924), from which seed was taken	Percentages of infection on sorghum grown from seed inoculated with—											
		Ordinary covered kernel smut						Smut strain from milo					
		Rosslyn, Va.		Dalhart, Tex.		Manhattan, Kans.		Rosslyn, Va.		Dalhart, Tex.		Manhattan, Kans.	
		1925	1926	1925	1926	1925	1926	1925	1926	1925	1926	1925	1926
Miscellaneous sorghums:													
White Yolo, Calif. Expt. Sta , 1924.		0	0.8	0	0	0	0	17.2	2.7	77.2	29.5	47.5	57.8
Darso	Plot 15	0	1.1	3.2	6.6	18.4	7.7	4.2	10.0	40.3	20.0	14.7	17.5
White durra, C. I. 81		7.1	14.9	38.3	27.8	53.4	40.4	10.3	15.2	31.3	40.0	31.5	48.3
Shallu, C. I. 85		24.6	12.5	28.7	16.8	73.0	89.9	14.6	0	47.8	40.1	46.6	30.2
Pierce kaferita, selection	Row 395 b	0	0	4.5	0	1.8	0	1.6	0	0	0	1.4	5.2
Do	Row 388 b	3.8	15.5	18.9		27.7	66.1	9.4	7.7	30.6		10.0	48.9
Freed Sorgo, head selection	Row 268	1.8	18.8	11.0	16.4	16.1	14.4	3.6	4.7	11.1	29.3	8.6	35.7
Weskan	Row 266	4.7	20.9	19.8	31.6	32.9	60.2	8.0	20.9	13.7	39.0	15.0	51.8
Shrock, selection	Plot 17	16.7	5.4	27.5	2.3	28.8	18.4	27.1	8.1	44.4	5.1	12.4	13.1
Premo, F. C. I. 8929			0				1.5		4.4				9.1
Chiltex, F. C. I. 8917			9.4				11.4		12.0				27.1

b 1923 seed.

The milos remained very highly resistant to, and most of them were immune from, the ordinary covered kernel smut. This is in agreement with the results of past experiments.[5] The milos were all more or less susceptible to the strain of smut occurring in milo and hegari. Juiciness and sugar content made little difference in the susceptibility of the selections. Yolo behaved very much as milo in that it showed very high resistance to the ordinary covered kernel smut, but proved susceptible to the strain from milo and hegari. It was even more susceptible to this form than most of the milos. The feteritas proved highly resistant to or immune from both strains of smut. In four instances, one at Dalhart and three at Manhattan, infection of feterita by the ordinary covered kernel smut occurred. The few cases in which smut occurred in milo and feterita grown from seed inoculated with the ordinary kernel smut undoubtedly are due to the presence of seed mixtures or natural hybrids. A great many such plants were cut out of the rows in 1925, but a few were still present in 1926. Wherever recognized, these plants were removed before the smut counts were made. A few of them, however, undoubtedly escaped notice. Open-pollinated seed was used in 1925 and self-pollinated seed in 1926. The few off-type plants which were found in the latter year undoubtedly were due to hybridization in previous years. One of the highest percentages of smut occurring in feterita (4.8 per cent) was found in a hybrid, F. C. I. 8926. One of the parents of this variety might have been a susceptible sorghum. In this case segregation might account for the result. It is not at all improbable that more than one strain of smut existed in the collection of the ordinary covered kernel smut, one being pathogenic to certain of the feteritas.

[5] REED, G. M., and MELCHERS, L. E. Op. cit.

Many of the Red Amber × feterita hybrids remained free or almost free from the ordinary strain of covered kernel smut, while a high percentage of them proved susceptible to the milo strain. Only two of these hybrids remained entirely free from both strains at all three stations.

Two of the three kafir × feterita hybrids proved susceptible to both smuts, while the third remained free from both strains at Rosslyn, Va., and Manhattan, Kans., in 1926.

The kafir × milo crosses all proved somewhat susceptible to both smuts. The same is true of the one milo × feterita hybrid. Kansas Orange × Dwarf Yellow milo and Blackhull × Sourless fall into the same class.

The two hegaris reacted in the same way as the milos, being highly resistant to the ordinary covered kernel smut but susceptible to the strain from milo and hegari.

In 1926 covered kernel smut which had been collected in 1921 at Chillicothe, Tex., by H. N. Vinall, on a new importation of feterita (S.P.I. 51989) from Shikaba, Sudan, was used to inoculate seed of a few varieties which was sown at Rosslyn, Va., and Manhattan, Kans. The results of this experiment are presented in Table 3.

The smut from feterita failed to produce smut in the three milos inoculated. Dwarf Sumac and broomcorn proved susceptible. The two feteritas, S. P. I. Nos. 51989 and 51991, became smutted when grown from 1925 seed which was inoculated with feterita smut. The results of tests with this collection of smut indicate that it is a different strain from the other two used in these experiments. Milo smut produced no infection in these varieties at either Rosslyn or Manhattan. Two heads in 106 of feterita, S. P. I. 51991, became smutted at Manhattan when grown from seed inoculated with the ordinary covered kernel smut.

The data in Tables 2 and 3 show that certainly two and probably three strains of covered kernel smut were used. The strain occurring on milo and hegari differs in its pathogenicity from the strain commonly occurring on sorghum, in that it infects milo and hegari about as heavily as it does the hitherto susceptible sorghums. From the meager data available the third strain on feterita appears to differ from the strain on milo in that it does not produce smut in milo but attacks two feteritas which are not smutted by the strain from milo.

TABLE 3.—*Reaction of varieties and strains of feterita, milo, sorgo, and broomcorn to a strain of covered kernel smut from feterita, when the plants were grown from artificially inoculated seed at Rosslyn, Va., and Manhattan, Kans., in 1926*

Group and variety, strain, or selection	Percentage of infection at—	
	Rosslyn, Va.	Manhattan, Kans.
Feterita:		
S.P.I. 51989 (1921 seed)	0	
S.P.I. 51989 (1925 seed)	4.8	5.7
S.P.I. 51991 (1925 seed)	10.8	11.2
Selection (Clovis, N. Mex., 1924)	0	
Milo:		
White (Texas, 1924)	0	
Dwarf Yellow (Texas, 1924)	0	
Selection (Hays, Kans.)	0	
Sorgo:		
Dwarf Sumac (Texas, 1924)	19.7	
Broomcorn (Texas, 1924)	26.3	

MORPHOLOGIC CHARACTERS OF THE STRAINS OF SORGHUM KERNEL SMUT

The fungus causing kernel smut in milo and hegari probably is a strain of covered kernel smut, *Sphacelotheca sorghi.*[6] Considerable difficulty has been experienced in attempting to arrive at a definite conclusion regarding the identity of the organism. Specimens obtained from New Mexico and Texas in 1923 and later years bore some resemblances, both macroscopically and microscopically, to both the covered kernel smut, *S. sorghi* (fig. 1), and the loose kernel smut, *S. cruenta* (Kühn) Potter. The membranes enveloping the smut balls seemed in some cases to be more fragile than those of the covered kernel smut, but resembled somewhat in this respect those of loose kernel smut. A microscopic examination showed that the membranes were composed of two types of sterile cells. The elongated cells of small diameter, adhering in chains when the tissue is macerated, are typical of *S. sorghi.* The spherical cells, with a diameter about twice that of the spores, and adhering rather loosely in clumps or balls, are typical of *S. cruenta.*[7] The specimens from Texas had a predominance of cells of the *sorghi* type (fig. 1), while those from New Mexico seemed to have more of the *cruenta* type of sterile cells. These spherical cells, however, were somewhat smaller, on the average, than the sterile cells found in authentic specimens of *S. cruenta.* Milo grown on Arlington Experiment Farm in 1924 from seed inoculated with spores from New Mexico and Texas contained fairly high percentages of smut (Table 1). An examination of smut galls from these plants at the time the normal kernels were in the milk stage revealed only the elongated chainlike cells of the *S. sorghi* type, regardless of the source of the inoculum. After the plants were mature both types of sterile cells were found in the smut balls. An examination of authentic specimens of both loose and covered kernel smut revealed both types of sterile cells in almost every collection examined. The type supposedly characteristic of the given species always predominated. Smutted specimens of milo grown at Arlington Experiment Farm and of milo and hegari collected in New Mexico and Texas were sent to G. P. Clinton, who decided that they were covered kernel smut, *S. sorghi.* He concluded that the spherical sterile cells present were somewhat too small and did not aggregate in balls sufficiently to be typical of *S. cruenta.*

The feterita smut collected by H. N. Vinall in 1921 and used in the experiments in 1926 (fig. 2, A and B) is morphologically very similar to the form on milo and hegari and should be placed in the same species, *sorghi.*

In September, 1926, a field of Dwarf Yellow milo containing 15 per cent of kernel smut was found near Shafter, Calif. This smut (fig. 3, A) with the exception of one head (fig. 3, B) was typical of the smut occurring on milo in New Mexico and Texas (fig. 1). The one head mentioned above was more typical of the loose kernel smut of sorghum than of that occurring on milo. Some of the smut galls were very long. In some cases they reached a length of about 1.5 cm. (fig. 3, B). The columella was long and rather pronounced. The enveloping membranes were somewhat more fragile than those of covered kernel

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[6] TISDALE, W. H., MELCHERS, L. E., and CLEMMER, H. J. Op. cit.
[7] POTTER, A. A. THE LOOSE KERNEL SMUT OF SORGHUM Phytopathology 5:149–154, illus. 1915.
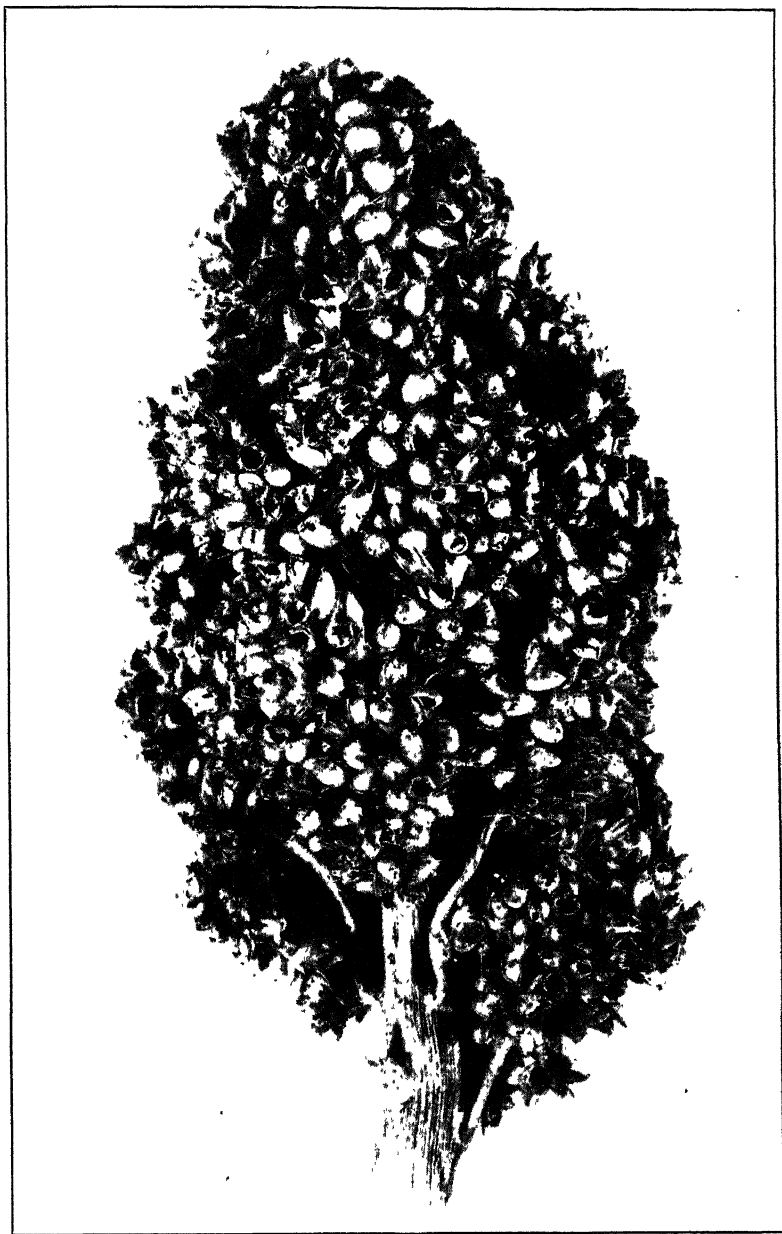
FIG. 1.—Kernel smut of Dwarf Yellow milo, from plant grown at Dalhart, Tex., in 1926. The smut apparently is a strain of covered kernel smut, *Sphacelotheca sorghi*

Fɪɢ. 2.—Kernel smut, probably a strain of *Sphacelotheca sorghi*, on feterita grown at Arlington Experiment Farm, Rosslyn, Va., in 1926: A, Feterita, S. P. I. 51991; B, Feterita, S. P. I. 51989
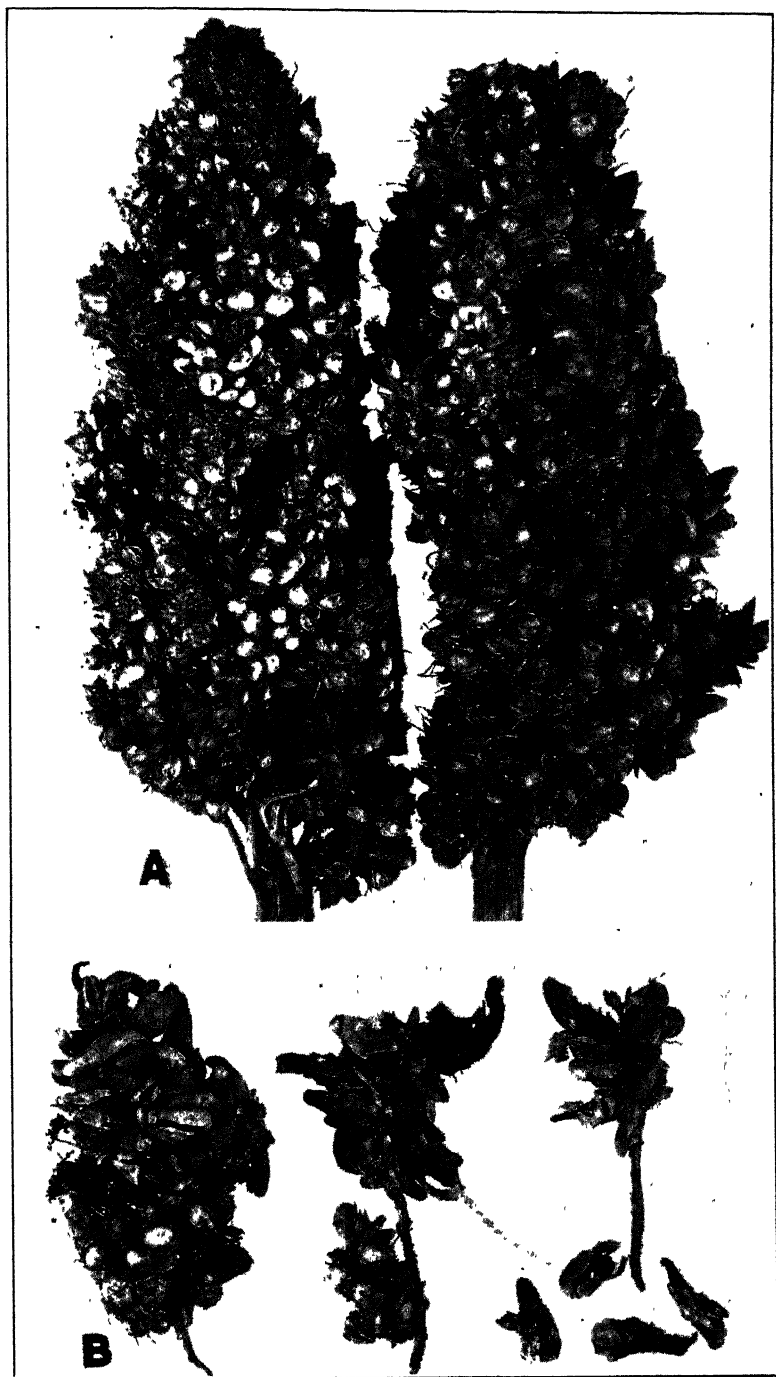
Fig. 3.—Kernel smut of Dwarf Yellow milo from different plants grown in the same field at Shafter, Calif., in 1926. These probably are variants within the strain of *Sphacelotheca sorghi*, which causes smut in milo and hegari: A, external characters typical of covered kernel smut, *S. sorghi;* B, external characters more like those of loose kernel smut. *S. cruenta*

smut, but not nearly so fragile as the membranes of loose smut. Both types of sterile cells were present, with the elongated chain-like *sorghi* type slightly predominating.

There seems to be more than one possible explanation for the occurrence in milo, hegari, and feterita of what appears morphologically to be intermediate stages between the two smut species, *Sphacelotheca sorghi* and *S. cruenta*.

In the first place, the peculiar strains of smut may represent hybrids between the two species. It is conceivable that a single sorghum plant might be infected by both fungi and that fusion might take place between growing hyphae of the two species, resulting in a hybrid fungus which might account for these variations and also for the apparently more virulent nature of the fungus which attacks the hitherto resistant sorghums. Fusion also may have taken place between sporidia of the two species on the surface of the seed and the resulting hypha may have infected the seedling and developed the hybrid form.

In the second place, there may be no sharp line of demarcation between *S. sorghi* and *S. cruenta*, as the descriptions in literature indicate. If a large number of collections of kernel smut were made from a large number of host varieties and under a wide range of climatic conditions throughout the world, it might be found that what has been described as two species simply represents the extremes in a series of variants. The strains in milo and feterita may represent hitherto unrecognized intermediates in such a series.

In the third place, these morphological variations from *S. sorghi*, the more closely related species, may be due to reactions of the particular host on the fungus. Busse[8] has stated that certain characteristics of the covered kernel smut are different on different host varieties. Potter[9] found the same to be true of loose smut.[10] They may simply be physiological variants within the species *S. sorghi* which are influenced by the host. This is perhaps the most probable explanation of the peculiarities in the morphology of these strains. While the morphologic differences should receive consideration, emphasis should be placed on the significance and importance of the physiologic differences which are reflected by the pathogenicity of these smut strains.

LOOSE KERNEL SMUT IN FETERITA

In addition to the collection of what appears to be a strain of covered kernel smut in feterita, the results of experiments with which are given in Table 3, J. H. Martin, Associate Agronomist in charge of Grain Sorghum Investigations, Office of Cereal Crops and Diseases, and G. T. Ratliffe, Superintendent of the United States San Antonio (Texas) Field Station, collected several heads of feterita, C. I. 182, from secondary shoots from stubble, which were smutted with kernel smut at San Antonio, Tex., in 1926. According to Ratliffe the heads produced on the primary culms were smut free. These

[8] BUSSE, W. UNTERSUCHUNGEN ÜEBER DIE KRANKHEITEN DER SORGHUM-HIRSE. Arb. K. Biol. Gendht. samt., abt. 4: 319–426, illus. 1904.
[9] POTTER, A. A. Op. cit.
[10] REED, G. M., and MELCHERS, L. E. Op. cit.
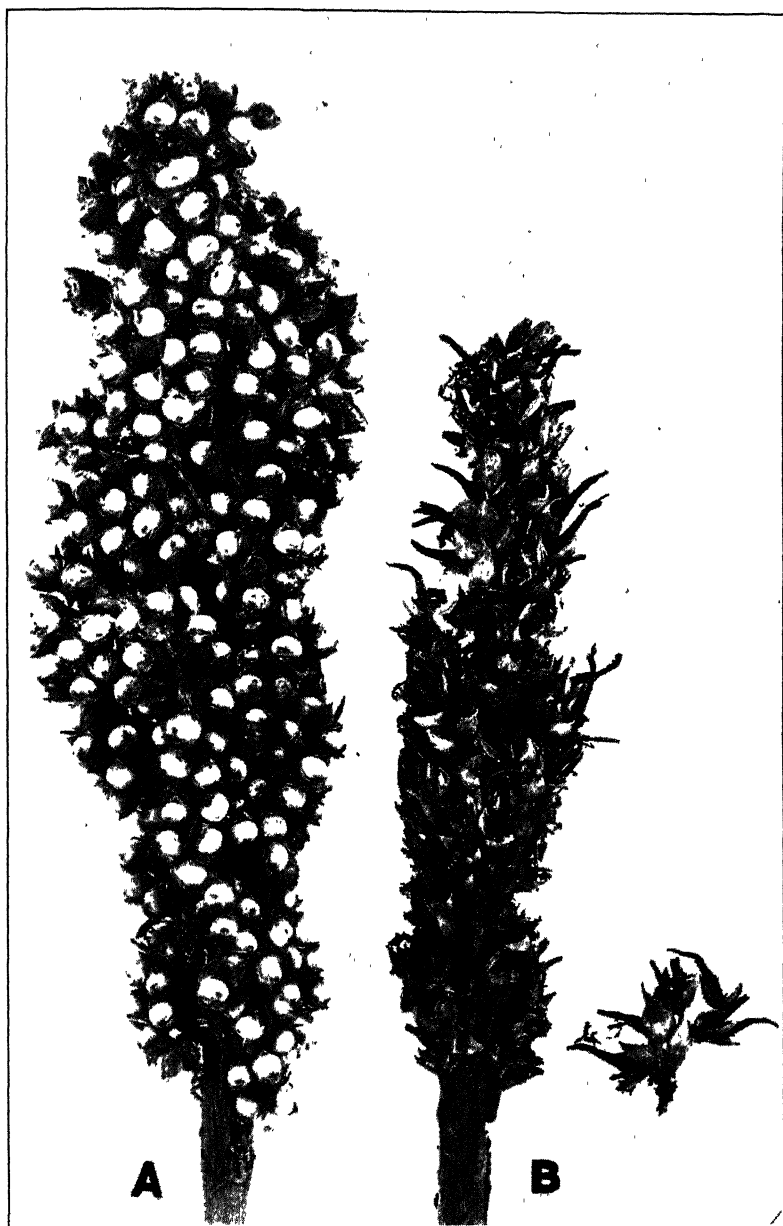
FIG. 4.—Feterita, C. I. 182. Heads from secondary shoots of plants grown at San Antonio, Tex. The primary heads, which were harvested early, were smut free: A, normal head; B, head smutted with loose kernel smut, *Sphacelotheca cruenta*

specimens were typical of the loose kernel smut, *Sphacelotheca cruenta* (fig. 4, B). This is interesting in view of the fact that Reed[11] found feterita to be resistant to both loose and covered kernel smut. The strain of feterita in which this smut was found was not included in his experiments, however. The occurrence of loose smut in feterita may be an indication that it is a special strain of *Sphacelotheca cruenta*, pathogenic on feterita. Again it may mean simply that infection takes place ordinarily when the smut spores are on the seed, but that the fungus is not able to keep pace with the growth of the plant unless the latter be cut back or injured in some other way to cause the production of secondary shoots, which are not able to outstrip the development of the fungus and consequently become visibly infected. Study is needed to clear up some of these points.

SUMMARY

In 1923 kernel smut was found occurring in milo in northwestern Kansas, northeastern New Mexico, and northwestern Texas. The smut also occurred in hegari in the same portions of New Mexico and Texas.

Preliminary experiments conducted in the greenhouse and field at Arlington Experiment Farm, Rosslyn, Va., in 1923 and 1924, showed in some cases high percentages of kernel smut in milo grown from seed inoculated with smut from milo.

Experiments were conducted at Rosslyn, Va., Dalhart, Tex., and Manhattan, Kans., in 1925 and 1926 to determine the reaction of numerous varieties and hybrids of sorghum to infection by the strain of kernel smut occurring on milo, and by the ordinary covered kernel smut of sorghum, *S. sorghi*. These proved that the smut occurring on milo and hegari differs from the ordinary covered kernel smut in that it is pathogenic on milo, White Yolo, and hegari, heretofore resistant sorghums. It also is pathogenic on the sorghums which are susceptible to the ordinary covered kernel smut.

Externally the smut on milo and hegari bears close resemblance to covered kernel smut, *Sphacelotheca sorghi*, although it occasionally is found to have certain of the characteristics of loose smut, *S. cruenta*. In almost every specimen, microscopic examination revealed two types of sterile membrane cells, namely, (1) the elongated cells of small diameter, occurring in chain fashion, typical of the sterile cells of *S. sorghi*, and (2) the spherical cells of larger diameter, grouped together in balls, and typical of the sterile cells of *S. cruenta*.

In all probability, the smut in milo and hegari is a strain of *Sphacelotheca sorghi*, although there may be some possibility that it is a hybrid between *S. sorghi* and *S. cruenta*, resulting from a fusion between hyphae of the two species growing in the same host plant, or the milo smut may be an intermediate in a series of variants ranging from *S. sorghi* as one extreme to *S. cruenta* as the other.

A collection of kernel smut of feterita made by H. N. Vinall in 1921 apparently is a strain of *Sphacelotheca sorghi*, differing in its pathogenicity from the ordinary covered kernel smut and also from the strain in milo and hegari.

Loose kernel smut, *Sphacelotheca curenta*, was found in secondary heads of feterita, C. I. 182, at San Antonio, Tex., in 1926. Feterita hitherto has been free from loose kernel smut.

[11] REED, G. M. VARIETAL RESISTANCE AND SUSCEPTIBILITY OF SORGHUMS TO SPHACELOTHECA SORGHI (LINK) CLINTON AND SPHACELOTHECA CRUENTA (KÜHN) POTTER. Mycologia 15: 132–143, illus. 1923

ANTHELMINTIC PROPERTIES OF SANTONIN [1]

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INTRODUCTION

In the world of therapeutics certain drugs or preparations have been used for so many years that the statements in the literature, especially in the textbooks and also in articles in professional journals, as to the action of these drugs have a tendency to reiterate impressionistic ideas or traditions. Printed statements are ordinarily copied and passed on with little or no attempt to substantiate the claims made. With a widespread impression once established it is difficult to revise opinions with regard to drugs and a writer is placed in a precarious position if his results conflict with the generally accepted views.

WIDE VARIATIONS IN METHODS OF ADMINISTRATION

Santonin is one of the drugs having an extensive traditional reputation as an anthelmintic. Its use and results have been varied in the hands of the users as reported by them. After many years of use the method of administration and the dose rate of the drug still show wide variations. It is recommended by some persons who have used it as valuable in single-dose treatments and by others as valuable only in divided doses continued over a considerable period. By some it is administered alone as a pure drug and by others combined with one or more substances said to have synergistic value. Some administer santonin simultaneously with a purgative; others regard this as bad practice and prefer to follow it with a purgative several hours after administration, while still others give a purgative both preceding and following the drug. The type of purgative advised is not a matter of uniform opinion, and various substances, such as salines, oils, calomel, and arecoline, have been advocated. Some believe that feed should be withheld from the subject preceding the treatment, while others make no such provision; some administer the preparation mixed in the feed, while others claim that this procedure gives very poor results. Certain workers insist on the necessity of individual treatments, while another group advises its use in flock treatments.

The great safety of santonin has repeatedly been referred to as one of the outstanding qualities of the drug. This is a factor of considerable importance when it is necessary to treat very young or weak animals for intestinal parasitism. However, to be useful, an anthelmintic must combine a fair degree of efficacy and cheapness with reasonable safety.

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[1] Received for publication Mar. 19, 1927; issued June, 1927.

SOURCES OF SANTONIN

The differences in results obtained are sometimes attributed to differences in the sources or nature of the santonin used. Recently certain commercial concerns have published the information that "that plant grows only in the wildest part of the Russian steppes, far from any seaport or commercial center, the most important area being the northeastern district of the Province of Turkestan." In this connection it may be noted that the plant from which santonin is obtained has been introduced into the United States and that it appears to thrive well in several semiarid sections of this country. While Turkestan is probably the native home of this plant, the Bureau of Plant Industry has grown it in an experimental way in several sections of the western slope of the United States. This fact, together with the reported increased foreign production, will probably operate to keep the cost of santonin at a reasonable figure in the future. The precise value of the domestic santonin has not been investigated by the writer, and in order to test the drug the Turkestan product was used.

EXPERIMENTAL WORK

A consideration of the writings of various workers shows a considerable divergence of opinion as to the value of santonin. The differences of opinion arise in large part from the fact that no uniform method of testing the drug has been used by investigators and that in only a few cases have accurate counts been made of the worms passed and a critical evaluation made of the results as ascertained by post-mortem examination of the animals treated.

In the present instance an effort has been made to get additional and definite information regarding the efficacy of santonin when used for anthelmintic purposes. A sample of the product with a guaranty of purity was procured from a representative of a firm interested in promoting its use. This was tested for purity in the Biochemic Division, Bureau of Animal Industry, and found to be free from adulteration. In order to get the most complete data possible it was used with and without purgation in varying doses, the drug being placed in hard gelatin capsules for administration. The dogs used in the experiment were confined in separate iron cages, were allowed their customary rations, and were given attention after treatment.

TESTS ON DOGS

FIRST SERIES

In the first set of experiments with dogs the animals were made to fast for 24 hours and santonin was given in the doses and with the results indicated below:

Dog 1060, weight 28 pounds; 1 grain santonin; worms passed, none; post-mortem, fifth day, hookworms 49, tapeworms 4, whipworms 7.

Dog 1059, weight 38 pounds; 2 grains santonin; worms passed, none; post-mortem, fifth day, hookworms 20, tapeworms 2, whipworms 7.

Dog 1057, weight 40 pounds; 3 grains santonin; worms passed, whipworms 6; post-mortem, fifth day, hookworms 31, ascarids 11, tapeworms 9, whipworms 38.

Dog 1065, weight 35 pounds; 4 grains santonin; worms passed, none; post-mortem, fifth day, hookworms 46.

Dog 1055, weight 33 pounds; 5 grains santonin; worms passed, none; post-mortem, fifth day, hookworms 16, tapeworms 17, whipworms 5.

Dog 1067, weight 39 pounds; 6 grains santonin; worms passed, none; post-mortem, fifth day, hookworms 2, tapeworms 8, whipworms 27.

From a review of these results it is evident that santonin used alone on dogs which have fasted for 24 hours and not followed by a purgative is not effective in the doses given. In only one instance did it show any anthelmintic action and in that case, dog 1057, 6 whipworms were passed and 38 remained. There was therefore an efficacy of 14 per cent against whipworms. Santonin gave no evidence of anthelmintic action in doses of from 1 to 6 grains in dogs harboring hookworms, ascarids, and tapeworms, and failed to remove any whipworms from 4 of the 5 dogs infested. The failure of 3-grain doses to remove ascarids is especially significant.

Second Series

In the following tests the animals were kept without food for 24 hours, and were given santonin and calomel simultaneously in the doses and with the results as shown below:

Dog 1063, weight 10 pounds; 6 grains santonin and 5 grains calomel; worms passed, whipworms 1; post-mortem, seventh day, tapeworms 7, whipworms 1.

Dog 1064, weight 18 pounds; 7 grains santonin and 5 grains calomel; worms passed, whipworms 4; post-mortem, seventh day, hookworms 10, tapeworms 8, whipworms 4.

Dog 1061, weight 35 pounds; 8 grains santonin and 5 grains calomel; worms passed, whipworms 1; post-mortem, seventh day, negative.

Dog 1066, weight 35 pounds; 9 grains santonin and 5 grains calomel; dog vomited first day; worms passed, none; dog died sixth day; post-mortem, no worms; post-mortem changes so extensive at necropsy that ante-mortem pathology could not be ascertained.

Dog 1058, weight 14 pounds; 10 grains santonin and 5 grains calomel; worms passed, whipworms 11; post-mortem, seventh day, hookworms 4, ascarids 1, tapeworms 1. (*Dirofilaria immitis* in heart.)

Dog 1060, weight 41 pounds; 11 grains santonin and 10 grains calomel; worms passed, hookworms 2; post-mortem, seventh day, hookworms 10, ascarids 1 (young), whipworms 5.

Dog 1056, weight 42 pounds; 12 grains santonin and 10 grains calomel; worms passed, whipworms 120; post-mortem, seventh day, hookworms 23, whipworms 8.

In this series of dogs, six of which had worms, santonin with a purgative showed an efficacy, against whipworms, of 100 per cent in two cases, 95 per cent in one case, and 50 per cent in two cases, for the five dogs infested with these worms. In only one instance did it remove any hookworms from the four dogs infested and in this case it expelled 2 of the 12 present, or 17 per cent. No efficacy was indicated in its action against ascarids or tapeworms, in spite of the fact that the doses used, from 6 to 12 grains, are much higher than those usually recommended. The death of one animal, dog 1066,

6 days after dosing can not be ascribed definitely to the treatment, as other dogs receiving the larger quantities were not affected. No symptoms other than a subnormal appetite and apathy were observed in this dog.

THIRD SERIES

In the next series of tests an ounce of castor oil was given in the morning 15 hours after a dose of santonin administered the previous evening. The dogs had fasted 24 hours. The results of this test follow:

Dog 1099, weight 6 pounds; 1 grain santonin; worms passed, none; post-mortem, fifth day, hookworms 4, ascarids 3, tapeworms 23, whipworms 6.

Dog 1098, weight 6 pounds; 2 grains santonin; worms passed, ascarids 7; post-mortem, fifth day, hookworms 2, ascarids 2, tapeworms 12, whipworms 3.

Dog 1085, weight 6 pounds; 3 grains santonin; worms passed, ascarids, 1; post-mortem, fifth day, hookworms 7, ascarids 22, whipworms 87.

Dog 1087, weight 15 pounds; 4 grains santonin; worms passed, none; post-mortem, fifth day, hookworms 31, ascarids 1, tapeworms 5, whipworms 7.

Dog 1070, weight 19 pounds; 5 grains santonin; worms passed, ascarids, 1; post-mortem, fifth day, hookworms 1, tapeworms 16, whipworms 2.

Dog 1103, weight 24 pounds; 6 grains santonin; worms passed, ascarids, 1; post-mortem, fifth day, hookworms 19, ascarids 2, tapeworms 18, whipworms 19.

The result of this test shows that santonin in the doses given followed by castor oil in 15 hours failed to remove any hookworms, tapeworms, or whipworms, and that its action against ascarids was not dependable. In two of the six infested inimals it failed to remove any ascarids and in the other four it was 100 per cent, 77 per cent, 33 per cent, and 4 per cent effective, respectively. These findings can not be correlated with the quantities given.

FOURTH SERIES

The same dose of santonin was given to the dogs in this test in addition to an increased amount of castor oil (2 ounces) at a shorter interval, 8 hours, the dogs having fasted 24 hours, with results as noted below:

Dog 1083, weight 6 pounds; 1 grain santonin; worms passed, hookworms 5, ascarids 8; post-mortem, fifth day, hookworms 112, ascarids 43, whipworms 8.

Dog 1100, weight 8 pounds; 3 grains santonin; no worms passed; post-mortem, fifth day, hookworms 8, tapeworms 4, whipworms 9.

Dog 1102, weight 13 pounds; 4 grains santonin; no worms passed; post-mortem, fifth day, hookworms, 4.

Dog 1086, weight 23 pounds; 5 grains santonin; worms passed, ascarids, 1; post-mortem, fifth day, tapeworms 3, whipworms 4.

Dog 1104, weight 32 pounds; 6 grains santonin; no worms passed; post-mortem, fifth day, hookworms 41, ascarids 15, tapeworms 5, whipworms 5.

In this group of animals there was no indication that the increased and earlier purgation gave better results. In one out of five animals infested with hookworms it removed 5 of the 117 worms present, or about 4 per cent. In two of the four animals infested with ascarids it was 100 per cent effective, in one case it was 16 per cent effective, and in one it failed completely. It likewise failed to remove any tapeworms from three infested dogs or whipworms from five infested dogs. A consideration of the effect of the treatment in the case of one dog, No. 1084, illustrates the manner in which anthelmintics may be misjudged in the absence of post-mortem examination of test animals. The passage of eight ascarids and five hookworms would be sufficient to convince some observers of a high efficacy of the drug. However, a comparison of the number passed with the total number of parasites present shows an efficacy of only 16 per cent against ascarids, 4 per cent against hookworms, and 0 per cent against whipworms.

SUMMARY OF TESTS ON DOGS

Santonin, without purgation and in doses varying from 1 to 6 grains administered to dogs weighing from 28 to 40 pounds, removed 6 of the 90 whipworms present, or about 7 per cent, but had no effect on the hookworms, ascarids, or tapeworms.

In doses of from 6 to 12 grains administered simultaneously with 5 to 10 grains of calomel to dogs weighing from 10 to 42 pounds, santonin removed 2 of the 49 hookworms, or about 4 per cent, 136 of the 154 whipworms, or 88 per cent, but none of the ascarids or tapeworms.

In doses of 1 to 6 grains, followed by 1 ounce of castor oil administered 15 hours later to dogs weighing from 6 to 24 pounds, santonin removed 10 of the 40 ascarids, or 25 per cent, but had no effect on the hookworms, tapeworms, or whipworms.

In doses of from 1 to 6 grains with 2 ounces of castor oil administered 8 hours later to dogs weighing from 6 to 32 pounds, santonin removed 5 of the 179 hookworms, or 3 per cent, and 14 of the 72 ascarids, or 19 per cent, but none of the tapeworms or whipworms.

TESTS ON SWINE

Two pigs weighing 78 and 81 pounds, respectively, were given 5 grains of santonin each in a light feed after having fasted for 12 hours. Six days later this treatment was repeated. It was not feasible to examine the feces of these animals, but on post-mortem examination 15 ascarids were found in one and 68 ascarids in the other. The dosing of animals by mixing the medicinal agent with the feed has never been regarded as an economical or scientific method of treatment by the skilled veterinarian. Certainly in most cases the efficacy of the drug is lowered by the process.

Following this test three pigs, each weighing between 30 and 40 pounds, were given capsules containing 5 grains of santonin after they had fasted for 24 hours. This was administered in the evening and on the following morning, about 15 hours later, and two hours before the regular mash feed was given, a small quantity of mill feed saturated with water in which 5 ounces of Epsom salt had been dissolved was given them. Purgation resulted and 4 ascarids were

passed during the next two days. Post-mortem examination of the animals one week later showed 59, 8, and 37 ascarids, respectively.

One animal weighing 45 pounds, which had fasted 24 hours, was then given 6 grains of santonin in a capsule, followed in 12 hours with a purge of Epsom salt. Two ascarids were passed following the treatment. Post-mortem examination of the animal on the sixth day showed 45 ascarids present.

To another animal weighing 36 pounds which had fasted 24 hours a dose of 15 grains of santonin in capsule was administered, followed immediately by 10 grains of calomel. The animal passed 16 worms during the three succeeding days. Post-mortem examination on the seventh day showed four ascarids in the small intestine.

SUMMARY OF TESTS ON SWINE

Summarizing the santonin tests on swine it may be stated that the dosage of 5 grains in feed was of little or no value. While there is a possibility that some worms were passed, the retention of 15 and 68 worms after treatment is sufficient to condemn either the drug or the method of administration. Santonin administered in 5-grain doses to animals weighing 30 to 40 pounds after they had fasted 24 hours, followed by a purge in 15 hours, removed 4 worms from a lot of three pigs and left an aggregate of 104 in the lot. A dose of 6 grains administered after a fast of 24 hours and followed in 12 hours by a purge of Epsom salt, proved insufficient for a 45-pound animal, removing only 2 of the 48 worms present. A dose of 15 grains accompanied by 10 grains of calomel administered to a 36-pound pig which had fasted 24 hours, proved most effective, removing 16 of the 20 worms present in the animal.

TEST ON HORSES

Many writers have recommended santonin for freeing horses of worms. One writer states that an animal given the classical carbon bisulphide treatment for bots followed in two weeks with a dose of from $\frac{1}{2}$ to $1\frac{1}{2}$ drams of santonin and an aloetic purge 12 hours later, may safely be pronounced worm free. This procedure was followed, and the maximum dose of $1\frac{1}{2}$ drams was given. Post-mortem examination 8 days later showed a great many Habronema of two species and some *Trichostrongylus axei* in the stomach. The intestine contained many cylicostomes of undetermined species and 132 large strongyles representing three species. Just what worms might have been removed is not known, but since so large a number of different varieties remained after the treatment it was not considered worth while to make further tests. No pinworms were found.

It should be mentioned in passing that the treatment suggested is not in line with what is known about the action of santonin and of carbon bisulphide. Santonin is rated generally as primarily valuable in removing ascarids, but carbon bisulphide has been shown by critical test to be approximately 100 per cent effective in removing ascarids from the horse, in addition to being equally effective in removing bots, which are not affected by santonin. There is, therefore, no point in following carbon bisulphide with santonin. It can be followed to advantage by chenopodium to remove the strongyles, cylicostomes, and pinworms from the large intestine.

CONCLUSIONS

The findings reported by Hall and Foster [2] that santonin in single doses of from 1 to 3 grains gave about 24 per cent efficacy in removing ascarids from dogs, and those of Mote [3] to the effect that a value of 0 to 46 per cent appears to be within the actual range of utility for the drug in removing ascarids from swine, are in substantial agreement with the findings reported in this paper. All critical testing of which the writer is aware shows an anthelmintic value for santonin much below what has been and is now being claimed for it in advertisements and in papers based on impressions gathered solely from seeing worms passed. If accurate data were obtained on the value of santonin as an anthelmintic by systematic post-mortem examination of test animals, much of the misinformation concerning its value could be eliminated.

The ease of administration of santonin is an advantage and may tend to prejudice the minds of users against other drugs more difficult to use in favor of santonin, and while it has value, especially in repeated treatments, it is a very much overrated drug at present. In the opinion of the writer its value is principally that of a drug which does not cause irritation of the gastro intestinal tract and hence is of value for use in repeated doses in cases of gastroenteritis with ascariasis, or in repeated doses for removing whipworms.

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[3] MOTE, D. C. ANTHELMINTIC EXPERIMENTS WITH HOGS. Ohio Agr. Expt. Sta. Bul. 378, pp. 153-182. 1924.

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THE OVIPOSITION RATE OF THE GRAPE LEAF HOPPERS [1]

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INTRODUCTION

The oviposition of insects has not yet been subjected to exact quantitative analysis. Ample evidence of this is to be found in the review by Richardson (*6*).[2] Before such an analysis can be profitably undertaken, the environmental factors conditioning productivity must be more clearly isolated. Data permitting such treatment have been obtained in the course of an investigation of embryonic and nymphal development of the grape leaf hoppers conducted at the bureau field laboratory at Sandusky, Ohio. These data are insectary data, and because of the fact that the insectary is of the small screened-cage type common in field work, the insectary thermograph records have been considered adequate for the purpose for which they have been here used.

MATERIAL AND METHODS

Adult grape leaf hoppers were collected in the vineyards and confined, each species separately, in lantern-globe cages approximately 4 inches high by 4 inches in diameter, as shown in Figure 1. Each lot of adults was etherized daily and transferred to a fresh leaf. By making these transfers in the evening, any immediate effect of the ether upon oviposition was minimized, for it had previously been determined that 80 per cent of the eggs are deposited during daylight hours.

The females were counted at the beginning of an experiment and following this at intervals ranging from 5 to 15 days. The number that died each day was small and seldom could be attributed to the ether. The dead hoppers were removed just prior to the daily transfer and the numbers recorded. Theoretically, therefore, the number of hoppers in a recount should equal the original number minus the number dead; actually it was usually less than this, the

difference representing escaped individuals. When this discrepancy, reckoned as $\dfrac{\text{number escaped}}{\text{number of days} \times \text{number of survivors}}$, equalled or exceeded 7 per cent, the record was thrown out; the average inaccuracy in the records retained was 2 per cent. In estimating the number of females in a jar each day, the number escaped was evenly distributed over the period between counts. Since all jars in which the number of escaped hoppers exceeded 7 per cent were eliminated, this assumption was probably legitimate. The eggs are concealed under the epidermis of the leaf, and can not be counted. The laying rate is based necessarily, therefore, on the number which hatched, the nymphs being easily visible.
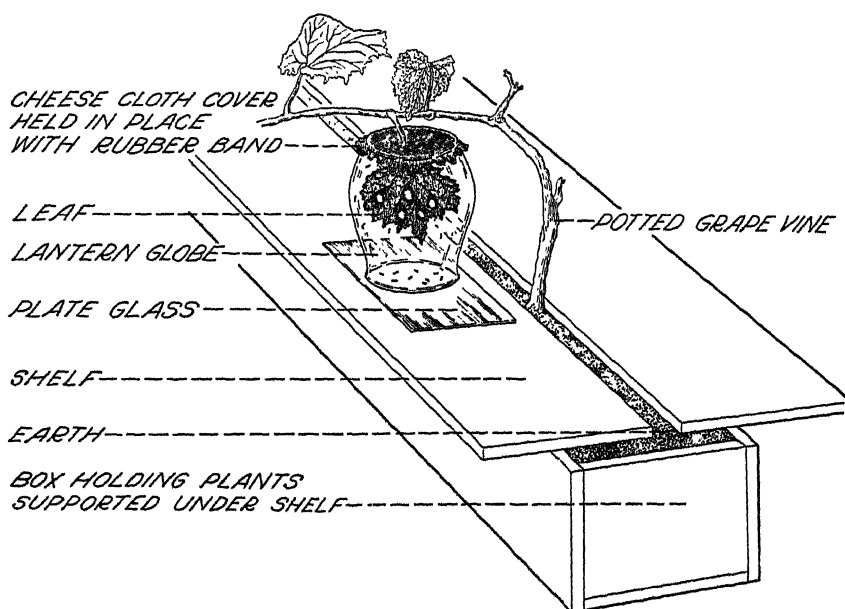
Fig. 1.—Lantern-globe cages used in the experiments with grape leaf hoppers

The adults employed were collected between June 18 and July 5, 1924, in vineyards near Sandusky, Ohio, and consisted largely, if not entirely, of overwintering individuals. The sex ratio, from 7 to 27 per cent males, was the ratio characteristic of the overwintering brood at this season. No mating was observed in any of the cages, nor did there seem to be any difference in laying rate per female per day between cages containing females only and those with the original percentage of males. The males, therefore, have been disregarded in the following analysis.

LAYING RATE

Of the five forms studied, three, *Erythroneura tricincta* Fitch var. *cymbium* McAtee, *E. comes* var. *comes* Say, and *E. comes* Say var. *compta* McAtee, occur commonly on thick-leaved grapes such as the

Concord (*Vitis labrusca*), but are also present in much smaller numbers on the thin-leaved Clinton grape, a variety which is almost pure *V. vulpina*, the native wild grape of the region. The other two species, *E. vitifex* Fitch and *E. vitis* Harris, show the opposite distribution (7). The average laying rates on the two species of grape in the insectary (Table 1) confirmed this specificity of response to host plant.

TABLE 1.—*Mean laying rate of five forms of leaf hoppers on different species of grape, in number of progeny per female per day*

Variety of grape	Species of leaf hopper				
	E. tricincta	E. comes	E. compta	E. vitifex	E. vitis
Concord_____	1.27±0.06	1.50±0.06	0.87±0.03	0.26±0.05	0.26±0.03
Clinton_____	.78± .06	.28± .03	.40± .03	1.17± .08	1.48± .11
r_____	ª.37, .03	ª.22, .41	ª.32, .09	---------------	ª.20, .36

ª Each correlation coefficient, *r*, between laying rate on Concord and laying rate on Clinton is followed by its corresponding value of *P*, as given by Fisher (*1*), rather than by its probable error.

This difference in laying rate was quite as marked during the first days in the history of a lot as at the end; the effect of the host plant was immediate and persistent. To a limited extent the variations in laying rate of the same species of hopper on the two species of grape have similar relations to the environment. The correlation coefficients of the laying rate on the thick-leaved grape with the laying rate for the same day on the thin-leaved grape varied from 0.20 to 0.37, but these are of small significance.

In general, the laying rate agrees with the proportionate representation in the field. Except in a small part of Middle Bass Island, Ohio, *compta* is comparatively uncommon, while *comes* and *tricincta*, which proved much more productive in the insectary, are quite generally the dominant forms in the Sandusky region. On wild grapes, *vitis* is uniformly the dominant species, and of the thick-leaved grape inhabitants, *tricincta* is to be found on wild stocks more often than either *comes* or *compta*; both these conditions agree with the productivity tests.

EFFECT OF TEMPERATURE

A change in temperature might conceivably affect the rate of oviposition in at least two rather distinct ways: (1) By direct effect upon the mechanism of egg deposition, and (2) by altering the rate of egg formation in the ovary. A correlation between the rate of egg laying and the temperature of the day the egg is laid might be attributed to either factor, but a correlation between rate and the temperature of the preceding day can be attributed only to the second factor. During the period covered by the oviposition records, June 18 to August 5, the daily mean temperatures, as recorded by the thermograph, varied from 17.4° to 29.2° C., and averaged 22.4°, with a standard deviation of 2.82°.

In Table 2 are given the original correlations of the zero order between laying rate and temperature. Of more importance, how-

ever, are the partial correlations of the third order, by which means spurious effects of the other measured factors may be eliminated. In this case $r_{1a\,234}$ gives the net correlation between the laying rate (a) and the mean temperature of the day the eggs were laid (1) when the composite effect of the temperature of the preceding day (2), of the number of females per jar (3), and of the age of the females from the date of the first record (4) are eliminated. Similarly $r_{2a.134}$ is the partial correlation between laying rate (a) and temperature of the preceding day (2) when the other three factors (1), (3), and (4) are hypothetically made inoperative.

TABLE 2.—*Correlations between laying rate (a) of five forms of leaf hoppers and environmental factors of temperature (1 and 2), number of females per jar (3), and date (4); the first three species on Concord, the last two on Clinton grape*

Correlation coefficients	Species of leaf hopper				
	E. tricincta	E. comes	E. compta	E. vitifex	E. vitis
Original:					
r_{1a}	0.58	0.40	0.30	0.04	0.50
r_{2a}	.61	.48	.30	−.08	.55
r_{3a}	−.24	−.15	.23	.65	−.55
r_{4a}	.39	.12	−.13	−.66	.50
Partial:					
$r_{1a\text{-}234}$	.41, ª.01	.13, .34	.17, .18	.16, .45	.20, .34
$r_{2a\text{-}134}$	.42, ª.01	.30, .03	.26, .04	.03, .89	.29, .16
$r_{3a\text{-}124}$	−.09, .54	−.18, .18	.20, .11	.17, .42	−.24, .24
$r_{4a\text{-}123}$	.31, .04	.10, .47	−.32, .01	−.22, .29	−.06, .78
Multiple R	.67	.38	.49	.32	.43
Number of cases	48	60	68	27	28

ª Value of P less than 0.01. As in Table 1, each partial coefficient is followed by its value of P.

It is at once apparent that the indirect effect of temperature upon rate of egg formation is generally of greater importance within the temperature range represented than its specific action as an "external" factor upon egg deposition. As is apparent from the correlations of laying rate with temperature of the two days, *comes, compta,* and *vitis* fall in one group, *tricincta* in another, and *vitifex* in a third. The greater dependence of *tricincta* upon temperature (twice as great) in comparison with *comes* may be correlated with its more restricted distribution geographically.

DENSITY OF POPULATION

Pearl (5) has demonstrated a marked decrease in progeny per female per day in Drosophila when the number of females to the bottle is increased, while Huettner (2) found that unless eggs were deposited regularly, eggs of Drosophila were laid at all stages of embryonic development. The number of female leaf hoppers per jar varied from 13 to 152, averaging from 54 (*tricincta*) to 76 (*vitis*). Under the conditions of the experiment, however, the effect of crowding this number on a single leaf or part of a leaf was barely appreciable. The partial correlation coefficients measuring this effect ($r_{3a.124}$) are of little significance; in the three commoner species the coefficients are negative, in the other two positive.

Approximate rates of egg laying for an equivalent calendar period were calculated from the experiments on fecundity reported by Johnson (*4*) and by Jewett (*3*). Using two, three, or four over-wintering females per jar, Jewett found average rates of 1.2 for *comes*, 1.0 for *compta*, and 1.7 for *vitis* (species of grape not reported), rates of the same magnitude as those obtained here. With four single pairs in separate larger cages, Johnson obtained an average rate of about 2.3 for *comes*, for four pairs in a single cage an average of 1.9, and for nine pairs, also in a single cage, an average of 4.0. Certainly if there is an effect of density of population upon rate of laying under the experimental conditions so far employed, it is of very much smaller magnitude than in Drosophila populations.

SEASONAL CHANGES IN FERTILITY

Most, if not all, of the hoppers used were overwintering individuals, which laid eggs from June 18 to August 5, the mid-points of the records lying between July 10 and July 20 for the different species. Both Johnson and Jewett found a marked decrease in the number of offspring produced as the season progressed. In the present experiments, however, correlations of laying rate with the age of the females in days, numbered from the beginning of the experiments $(r_{4a.123})$, gave erratic results, varying from -0.32 (*compta*) to 0.31 (*tricincta*). That individual *tricincta* females actually laid more eggs as the season progressed is questioned, the significant positive correlation between laying rate and age in this species being attributed instead to a differential mortality. It is of interest, however, that the resultant effect of all factors involved in the seasonal change, as measured by the correlation coefficient, increases the productivity of *tricincta*, does not affect the oviposition of either *comes* or *vitis*, and decreases egg laying in the less abundant *compta* and *vitifex*.

THE TOTAL EFFECT OF ENVIRONMENT UPON LAYING RATE

The combined effect of all the environmental factors considered on reproductive rate upon a single species of host plant is given by the multiple correlation coefficient R, which may take any value from 0 to 1, and is given in Table 2. These vary from 0.32 (*vitifex*) to 0.67 (*tricincta*), and, except for *vitifex*, are all significantly different from 0. That no important environmental factor, common to all species, has been overlooked is indicated by three facts: (1) Correlations between laying rates on Concord and on Clinton on the same day are smaller than the multiple coefficients; (2) the correlation in laying rate between two lots of *compta* collected at the same time and place and covering 28 days of oviposition on Concord was 0.44 ($P = 0.02$), in agreement with the multiple correlation coefficient 0.49; (3) the correlation of laying rate of *comes* with laying rate of *tricincta* on the same day was 0.33 ($P = 0.03$), less than either multiple coefficient, these two species agreeing most closely in their partial coefficients.

In the best case, *tricincta*, the multiple correlation coefficient represents a control over laying rate of but 45 per cent. Accordingly, it would be futile to attempt to treat these data quantitatively. It is better to leave them in the qualitative form in which they have been presented.

SUMMARY

The productivity of grape leaf hoppers was investigated by means of the daily rate of oviposition by overwintering females. Of the five forms studied, *Erythroneura tricincta* var. *cymbium*, *E. comes* var. *comes*, and *E. comes* var. *compta* showed much greater productivity on *Vitis labrusca* stocks than on vines predominantly *V. vulpina*. *E. vitis* and *E. vitifex*, on the other hand, were more productive on *vulpina* stocks. Both of these conditions agree with the distribution of the respective species in the field.

The effect of different environmental conditions on laying rate was measured by partial-correlation methods. Of these environmental factors, temperature was found to condition oviposition more by its indirect effect upon egg development than by direct action on egg deposition. The effect of density of population was slight. The action of seasonal changes on rate of egg laying agreed with the relative abundance of the different species in the field. The total effect of the environment in these experiments, as measured by the multiple-correlation coefficient and other constants, was not large, but probably did not omit any important factor common to all the experiments.

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AN UNDESCRIBED WHITE FLY ATTACKING CITRUS IN PORTO RICO [1]

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As certain white flies are considered to be among the worst pests of citrus in various parts of the world, it is interesting to present here the following description of a new citrus-feeding species. Although at present not of economic importance in its native home in Porto Rico, it is of potential importance to other citrus-growing countries.

Paraleyrodes naranjae, new species

Very close to *Paraleyrodes goyabae*, a Brazilian species, but easily distinguished by the difference in male genitalia.

EGG.—Length 0.240 mm., width 0.112 mm. Pale yellow in color. Scattered over leaf and lying rather prostrate, attached at one extremity to long pale stalk.
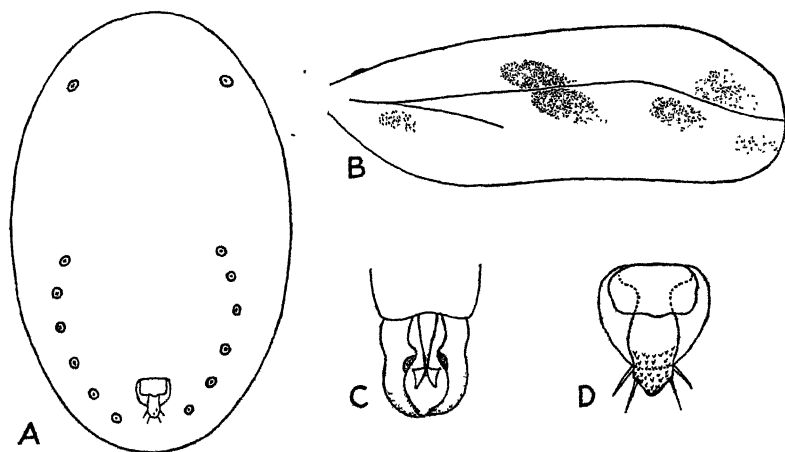
FIG. 1.—*Paraleyrodes naranjae;* A, pupa case; B, adult forewing; C, male genitalia; D, vasiform orifice

SECOND LARVAL INSTAR.—Distinctly orange-yellow, with a very short and fine fringe of waxy hairs around the margin.

THIRD INSTAR.—The waxy fringe is much shorter than in the fourth instar and the thick wax filaments arising from the dorsal pores have not become so thick and high, although hiding the body even in this stage.

FOURTH INSTAR (pupa case).—Occurs scattered over the under surface of the leaf as small white waxy rosettes that measure about 1.50 mm. across. Case fringed with very broad white waxen filaments that curl downward, the case being completely hidden by the other very broad thick filaments that arise from

the dorsal pores and grow upward, curving slightly. Size of case about 0.85 by 0.50 mm.

ADULT MALE.—Body uniformly pale yellowish with the claspers a very distinct red in freshly mounted specimens. Length from head to tip of claspers 1.14 mm. Antennae very peculiar, three-jointed; the first joint cup-shaped, whitish, length 0.04 mm., second joint subpyriform, whitish, length 0.05 mm., third joint, golden yellow, extremely long, 0.55 mm., very much imbricated, a few hairs at tip. Eyes distinctly constricted, dark reddish-brown. Fore wing 0.95 mm. by 0.40
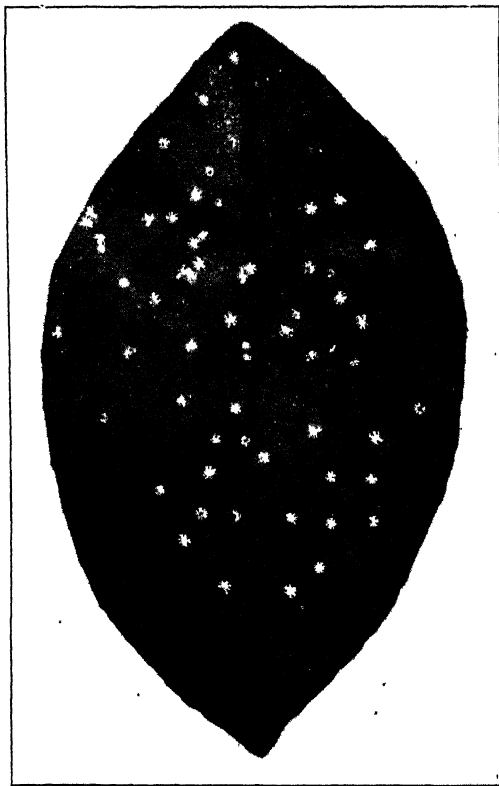
FIG. 2.—Sour-orange leaf infested with *Paraleyrodes naranjae.*
Natural size

mm., with the radial sector and a short media retained, dusky spotted as in Figure 1; hind wings with single straight vein. Genitalia forcipate, the claspers 0.11 mm. in length, stout at base and curving.

ADULT FEMALE.—Smaller than the male, length to tip of ovipositor 0.81 mm. Body pale yellowish white. Antennae differ from those of male, being distinctly four-jointed.

Described from numerous adults, pupa cases, et cetera, collected by the writer on old sour-orange trees (fig. 2) in back-yard garden of Señor Hernandez Lopez at car stop 23, Santurce, Porto Rico, December 21, 1924, on which date they were abundant in association with the woolly white fly, *Aleurothrixus howardi* (Quaint.).

Paraleyrodes naranjae in all of its stages, including the egg, lies amid very short fine broken waxen rods scattered indiscriminately over the lower surface of the leaf.

A single female parasite, *Encarsia variegata* How. (determined by Gahan), was reared from this material January 3, 1925. However, the species was very heavily parasitized. Thirty out of a total of 49 pupa cases mounted on slides, or 61.2 per cent, showed evidences
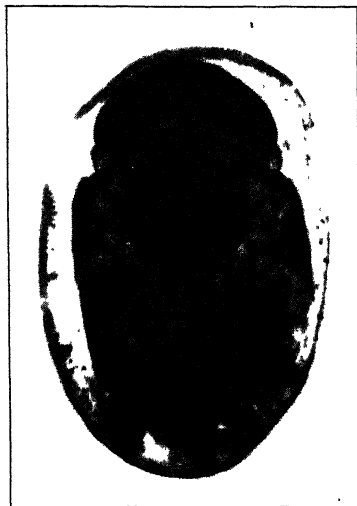
FIG. 3.—Pupa of the parasite *Encarsia variegata*, lying in the pupa case of the white fly. Greatly enlarged

of parasitism. A single pupa of the parasite almost completely fills the pupa case of the host (fig. 3). *Encarsia variegata* was described by L. O. Howard[2] as a parasite of *Paraleyrodes perseae* in Florida.

[2] HOWARD, L. O. ON TWO NEW SPECIES OF PARASITES OF ALEYRODIDÆ. Ent. Soc. Wash. Proc. 10: 64. 1908.

THE YELLOW RUST OF RASPBERRY CAUSED BY PHRAGMIDIUM IMITANS[1]

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INTRODUCTION

Although *Phragmidium imitans* Arthur is distributed from the Atlantic to the Pacific coast in the northern United States and southern Canada, it is not prevalent enough to be of economic interest except in the Pacific Northwest. Its geographical range is from Newfoundland and Massachusetts to Colorado and northern California, and northward into Canadian Provinces.

Phragmidium imitans occurs autoeciously on species of the genus Rubus, having been reported on *R. leucodermis* Douglas, *R. occidentalis* Linn., *R. spectabilis* Pursh, and *R. strigosus* Michx. The widest distribution is on the latter host, upon which it has been reported in Canada from Newfoundland and Nova Scotia to Ontario, and in British Columbia; and in the United States from Maine and Massachusetts to Montana and Colorado, and in Oregon and Washington. On the other species of Rubus, *P. imitans* is distributed as follows: On *R. leucodermis*, from northern California to British Columbia; on *R. occidentalis*, in Massachusetts, Newfoundland, Oregon, Washington, and British Columbia; and on *R. spectabilis*, in Oregon and Washington. The severest attacks are on *R. strigosus* and *R. spectabilis*.

TAXONOMY

The organism causing this disease was first described by Farlow[2] in 1879 as *Phragmidium incrassatum* Link *gracile* and later (1884) by Arthur[3] as *P. gracile*. Since the latter name was preoccupied (*P. gracile* Cook, 1871) and the fungus is similar to these other species, Arthur finally gave the name *P. imitans*.[4]

MORPHOLOGY

There is nothing strikingly distinctive in what is known of the morphology of *Phragmidium imitans*. It is very similar to the European species, *P. Rubi-Idaei* Karst. In *P. imitans* the pycnia, which are flattened lenticular bodies opening by irregular clefts through the overlying epidermis of the host, are typical of the genus. Under Oregon conditions they first appear on the upper surface of the leaves in April and early May. They are located at the centers of slightly elevated spots, occurring singly or more often in groups of two to four. These elevated spots are less than a millimeter in diameter,

Journal of Agricultural Research,
Washington, D. C.

Vol 34, No. 9
May 1, 1927
Key No. Oreg.-16

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and may be slightly lighter in color than the normal tissues of the succulent raspberry leaves. If it were not for the tiny circular elevations upon which the pycnia occur, they would pass entirely unnoticed at this stage. When mature, pycnia are more conical than lens-shaped and measure 45μ to 90μ in diameter and 30μ to 35μ high.

The tiny elevations upon which the pycnia occur are the unruptured, developing aecia. These break through the epidermis and become noticeable from late in April and early May until late in July. They rupture the epidermis in small circular groups around the pycnia, becoming pulvinate. In color they are a light orange yellow, fading to pale yellow. They may occur scatteringly or so densely spotting the leaves (fig. 1, A) that early defoliation results. The circular aecia (fig. 1, B) are surrounded by incurved clavate paraphyses just inside of the fringing epidermis. The paraphyses are 10 to 15×45 to 100μ, with thin, smooth, hyaline walls.

The aeciospores are globose to broadly ellipsoid, 16 to 24×14 to 20μ in size, with pale-yellow, sparsely and sharply papillose walls.

The uredinia have been found as early as the first of May, but are generally most prevalent in badly infected plantings during the month of June. Nevertheless, they may be found more or less abundantly until the leaves are shed in the late fall. Uredinia occur in lesions on the underside of the leaves and on all types of stems, including the canes (fig. 1, C, D, and fig. 2, C),[5] fruiting laterals (fig. 2, A), and leaf petioles (fig. 2, B). On the leaves the uredinial lesions are roundish, but on stem tissues they are usually elliptical. They soon become so prolific in spore production that the separate uredinia are indistinguishable. The uredinia are circular, small, 0.1 to 0.2 mm. in diameter, the ruptured epidermis disappearing early; paraphyses encircling the sorus are rather numerous, incurved, somewhat clavate, 65 to 100×11 to 17μ in size, with thin, colorless, smooth walls.

The urediniospores are broadly ellipsoid, 18 to 23×15 to 18μ in size, orange to pale yellow, becoming almost white late in the season, with thin walls, sparsely and rather strongly verrucose-echinulate, and with obscure germ spores.

The telia come in the uredinial lesions, wherever they occur. They have been found from the middle of July until late into the winter. In late summer the telia, blackened by the teliospores, are conspicuous on the lower surface of the leaves (fig. 2, D) and on the cane lesions until spring (fig. 2, C).

The telia which range from 0.3 to 0.7 mm. in diameter, are scattered. They are blackish, and become naked early.

The teliospores are cylindrical 26 to 32×80 to 127μ, in size usually rounded below; apex has a blunt, conical hyaline apiculus, 3μ to 13μ long which has from 6 to 10 cells (usually 8 or 9); wall very dark fuscous brown, closely and rather coarsely verrucose; pedicel rugose when dry, sometimes with a twisted appearance, 80μ to 120μ long, the upper portion colorless except near the spore, 9μ to 10μ in diameter, the lower portion colorless but the interior not clear, swelling in water to claviform, 14μ to 27μ in diameter.

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[5] FRANK, A. A NEW PHASE OF THE RASPBERRY RUST. Wash. Agr. Expt. Sta. Mo. Bul. 4 (10): 15–16. 1917.
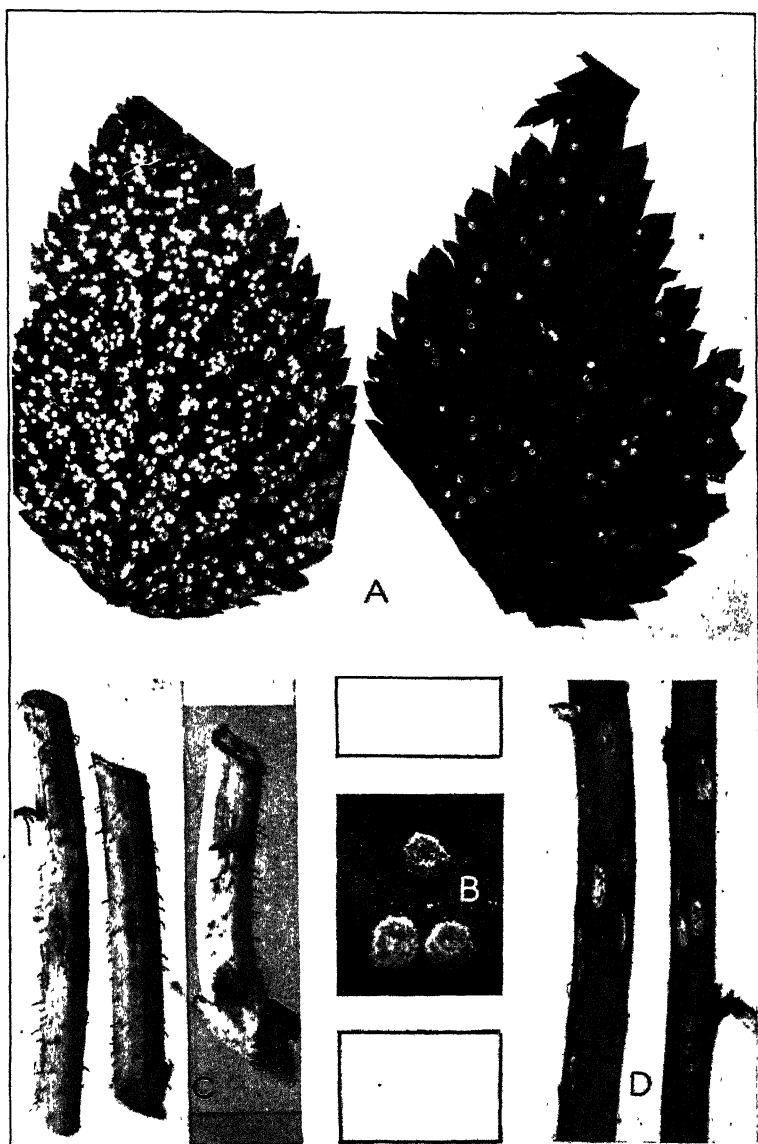
FIG. 1.—A, aecia on the upper surface of leaflets from the Cuthbert red raspberry Natural size. B, three aecial sori enlarged. ×10. C, uredinia on newly grown canes of the Cuthbert red raspberry, showing early stages of cane lesions beginning as dark water-soaked areas. Natural size. D, uredinial lesions on current year's canes late in the summer, showing an abundance of urediniospores. Natural size

FIG. 2.—A, uredinial lesions on fruiting laterals of Cuthbert red raspberry taken in July. Natura size. B, a uredinial lesion on leaf petiole. Natural size. C, uredinial lesions with telia appearin subsequently in the same lesions near the base or ground line on two canes in their second seasor Natural size. D, pale yellow uredinia and black telia on the lower surface of a Cuthbert leaf ✕

GERMINATION OF SPORES

No infection studies have been conducted, but several attempts have been made to germinate spores on microscope slides moistened with water. All attempts to germinate aeciospores have failed, but a very few teliospores have germinated and a considerable number of urediniospores. All attempts to germinate current season teliospores failed, but a very small percentage held over from the previous year have been induced to germinate in this way. Figure 3 shows a germinating teliospore of *Phragmidium imitans*, illustrating the type of promycelium produced and the subglobose sporidia which are hyaline and measure 4μ to 6μ in diameter. Figure 4 shows the germ tube of an urediniospore issuing from a terminal germ spore.
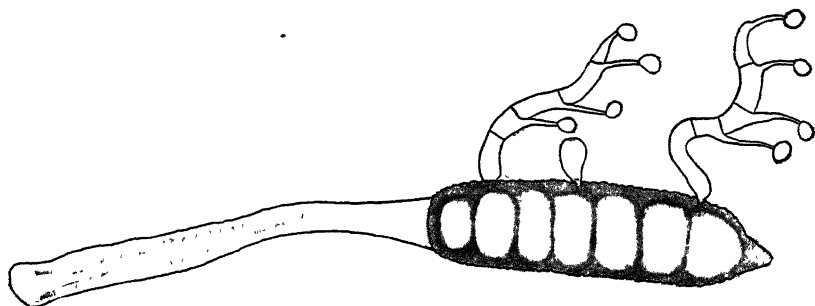
Fig 3.—Germinating teliospore. ×500

ECONOMIC IMPORTANCE

The disease of the Cuthbert red raspberry caused by *Phragmidium imitans* develops to damaging proportions in the Pacific Northwest, particularly in seasons when the spring rains continue late. Under such conditions leaf infections become so numerous (fig. 1, A) that partial defoliation in badly infected plantings is frequently observed, and often more severe defoliation is reported. Berry pickers fre-

Fig. 4.—Germinating uredinio-spore. ×500

quently have their clothing almost covered with the orange urediniospores. In seasons when leaf infections are so numerous as to produce any appreciable amount of defoliation the disease must have some devitalizing influence on the affected plants. However, the actual loss in crop during the current and subsequent years from this cause would be difficult to ascertain. The infection which causes uredinial lesions on the stems is the most serious and damaging phase of the disease.

UREDINIAL LESIONS ON CANES

The greatest economic loss from raspberry rust is caused by the uredinial lesions on the stems. Frank[6] first called attention to these cane lesions in 1917, mentioning the fact that the spores produced in these lesions are doubtless an important factor in the carrying over of

[6] FRANK, A.   Op. cit.

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the fungus from one season to another. In answer to the writer's inquiry H. S. Jackson states that at the time the original description of *Phragmidium imitans* was made stem lesions were not known but that several years ago he found this disease on stems from western Washington. Although these uredinial lesions on the stems have been known for some years it is only recently that they have received detailed attention by the writer. In the summer of 1923 several growers of Cuthbert red raspberries in western Oregon called the writer's attention to the fact that they were sustaining a considerable loss of canes from some cause unknown to them. An investigation led to the conclusion that the loss was directly attributable to cane lesions.

During the summer when infection of the canes takes place the lesions on the green succulent canes do not present a serious aspect except where the lesions are so close together and so arranged as almost completely to girdle the stem. Comparatively few canes wilt or die during their first year. Losses from rust usually occur during the second or fruiting year of the canes. The infections near the ground have become deep and cankerous by the second year. (fig. 2, C). If they are numerous, they not only hinder the normal rise of sap but also produce brittleness in the canes. Many of the brittle canes are accidentally broken off when the old fruiting canes are trimmed out, and many more are broken out when the canes are trellised. Frequently, also, berries dry on the bushes before they reach maturity. Canes with numerous lesions near the base seldom have enough vascular tissues remaining to allow the passage of the amount of sap necessary to carry the fruit to maturity. In such cases the fruit of the whole cane suffers, while in the case of infections on the fruiting laterals the fruit of the individual lateral is damaged. (fig. 2, A). Leaves having petiolar infections (fig. 2, B) usually wilt and dry up during the heat of the summer, particulary if they are borne on the second-year fruiting canes and the fruit robs them of some moisture. Not infrequently the cane lesions produced by *Phragmidium imitans* are subsequently infected with *Fusarium viticola* Thüm, in which cases the pinkish sporodochia give to the lesions the appearance of anthracnose spots.

CONTROL

No satisfactory control measures have been found for this yellow raspberry rust. If in badly infected plantings the old fruiting canes are removed as soon after the harvest of the fruit as practical, much of the source of late summer infection will be removed. Where practical, all the fallen leaves and refuse resulting from the removal of the old canes should be raked up and burned. Early spring plowing to cover fallen leaves and refuse before the leaves come out offers the most practical method of control. Immediately after plowing, the refuse should be raked into the furrows before the first cultivation. If these sanitary measures are thoroughly carried out the disease should never become serious, although such measures do not eliminate the possibility of infection from lesions on the new canes. In most seasons, however, such infections are practically negligible.

A few tests have been made to determine the effectiveness of Bordeaux mixture spray, 3-3-50 in preventing infection of the newly

growing canes.   One of these tests made in 1924 gave rather hopeful results.   The tests made in 1925 and 1926, however, were unsatisfactory as the seasons were not favorable to rust and the unsprayed rows showed no lesions on the new canes.   Three applications were given when the new canes were 4 to 12 inches, 16 to 20 inches, and 20 to 36 inches high, respectively.   In the 1924 tests the sprayed canes were not infected in stems of leaves, but the unsprayed rows showed some cane lesions and moderate leaf infection.   These results are merely indicative, since conditions in 1924 were not favorable for infection by *Phragmidium imitans*.   Grove [7] says that in England the spread of *Phragmidium Rubi-Idaei* Karst. is prevented by sprays such as dilute Bordeaux mixture or potassium sulphide solution.   A convincing test of Bordeaux spray as a preventive of *P. imitans* is impossible, however, in seasons as unfavorable to the disease as those of 1924, 1925, and 1926.

SUMMARY

In this paper is described the yellow rust of red raspberries caused by *Phragmidium imitans* Arthur.   The geographic distribution of the rust and the species and varieties of Rubus upon which it occurs are briefly reviewed.   The life history as observed on the Cuthbert red raspberry in the Northwestern Coast States is described, with particular reference to the uredinial lesions on the stems.   The infection of second-year or fruiting canes near the ground is the most serious phase of the disease, since the resulting lesions produce brittleness and diminish sap-conducting tissues.   Thus canes are easily broken in trellising, and the rise of sap may be insufficient for complete maturity of the fruit.

No satisfactory means of control are known, but extreme sanitary methods for the elimination of old leaf and cane refuse are the most practical methods suggested.

[7] GROVE, W. B.   THE BRITISH RUST FUNGI (UREDINALES) THEIR BIOLOGY AND CLASSIFICATION.   p. 299. Cambridge.   1913.

A COMPARISON OF THE DIRECT MEASUREMENT OF THE HEAT PRODUCTION OF CATTLE WITH THE COMPUTATION OF THE HEAT PRODUCTION BY THE RESPIRATORY-QUOTIENT METHOD [1]

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INTRODUCTION

The method of computation of the heat production of animals by the respiratory-quotient method—that is, from the carbon dioxide produced, the oxygen consumed, and the nitrogen of the urine—has seemed to require confirmation as applied to cattle, because of the modification of the standard procedure as employed with omnivora and carnivora which is necessitated by reason of the extensive fermentation of carbohydrates occurring in the alimentary tract of ruminants.

The present study was undertaken, therefore, to compare these computed values with direct measurements of the heat production by means of the respiration calorimeter of this institute—the only apparatus of the kind in existence of the size necessary for use with cattle. Obviously this comparison is of especial interest in relation to the heat-production values derived from the respiratory exchange as determined by means of the several respiration chambers which are being used in studies of the energy metabolism of cattle.

TABLE 1.—*Schedule of experiments*

Experiment No.	Animal	Period No.	Treatment
221 E	Cow No. 885	3	Fasting; sixth day (*5*). [a]
221 F	Cow No. 874	1	Supermaintenance (*4*).
221 F	Cow No 874	2	Maintenance (*4*).
221 F	Cow No. 874	3	Fasting; ninth day (*5*).
221 F	Cow No. 886	1	Supermaintenance; milk production (*4*).
221 F	Cow No. 886	2	Do.
221 F	Cow No. 887	1	Supermaintenance (*4*).
221 F	Cow No. 887	2	Maintenance (*4*).
221 F	Cow No. 887	3	Fasting; ninth day (*5*).
235	Steer No. 259	1a, 1b, 1c, 1d	Fasting; days 5 to 10½ (*3*).
235	Steer No. 259	2	Maintenance. [b]
235	Steer No. 259	3	Supermaintenance. [b]
235	Steer No. 260	1a, 1b, 1c, 1d	Fasting; days 4 to 9½ (*3*).
235	Steer No. 260	2	Maintenance. [a b]
235	Steer No 260	3	Supermaintenance [a b]

[a] Reference is made by number (italic) to "Literature cited," p. 878.   [b] Unpublished data.

Accordingly, quantitative determinations were made, in connection with three comparatively recent experiments, of the oxygen of the air coming from the respiration calorimeter chamber; the other data

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 9
May 1, 1927
Key No. Pa.-22

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used in the computation of the heat production by the respiratory-quotient method being regularly determined in providing for the confirmation of the direct-heat measurements by comparison with the heat production as computed from the balance of matter and energy.

The schedule of experiments discussed, including references to publications of the full accounts, comprises Table 1.

DISCUSSION OF METHODS OF EXPERIMENTATION AND OF DATA INVOLVED IN COMPUTATIONS

The direct measurements of the heat production which are involved in this comparison were made by the standard method followed at this institution from the time of inception of its program of research in animal calorimetry in 1902 to the present date. The main portion of the heat given off by the animal is directly measured in a stream of cooling water which flows through copper piping in the respiration chamber, the only other large portion of the heat produced being that used to vaporize the moisture given off from the animal's skin and lungs. The equipment provides for the direct weighing of this moisture, thus permitting the computation of the corresponding latent heat of water vapor.

In the indirect respiratory-quotient method of computation of the heat production the following data are involved: the carbon dioxide and oxygen of the ingoing and the outcoming air, the cumbustible gases (methane) of the outcoming air, and the nitrogen of the urine. Two procedures for dealing with the gases and heat of fermentation are compared, that of Andersen (1), and another in which the factors expressing the relation of the heat of fermentation to the methane produced, and the relation of the CO_2 to CH_4 of fermentation, were as recommended by Krogh and Schmit-Jensen (7). For the sake of brevity the second procedure is referred to as that of Krogh.

Since the composition of the air coming from the calorimeter varies much throughout the day, in accord with variations in the voluntary and the vital activities of the animal and with lapse of time since feeding, it is obvious that no system of intermittent sampling of the air can be depended upon accurately to represent the entire volume involved. Provision should be made, therefore, for the collection and analysis of a continuous aliquot of the air, the rates of ventilation and of sampling being maintained constant, or the rate of sampling being so controlled as to be accurately responsive to uncontrolled variations in the rate of ventilation.

In these experiments determinations of CO_2 were made in continuous aliquots of the air, as above specified, and also in intermittent samples taken at half-hour intervals; but oxygen was determined only in samples taken at half-hour intervals. The comparison to be made, therefore, between the direct and the respiratory-quotient methods of measurement of heat production should be considered in relation to the details of the particular procedures followed, which, in relation to the respiratory-quotient method, fail obviously to provide for the greatest accuracy of measurement practicably obtainable, even though the procedure followed probably compares favorably as to accuracy with the methods of most of the workers who utilize the respiratory-quotient method.

Whatever the facts as to the accuracy of the work by the respiratory-quotient method, and whatever the procedure—direct or indirect—which is followed, it is the feeling of the writers that it is of importance in studies of the utilization of feed energy by cattle that the utmost practicable nicety of procedure be employed.

Accordingly, in the light of the results of the preliminary studies here reported, this institute has undertaken to develop simple and accurate meter pumps, and accurately synchronous aliquoting spirometers for use with large farm animals and others for use with small laboratory subjects such as rats.

It is well known that the composition of the outside air is characterized, in general, by a high degree of constancy. It was not considered necessary, therefore, in the experiments here discussed to take as many samples of the ingoing as of the outcoming air. In the experiments with steers three samples of the outside air were taken during each 12 hours, and the average analysis for CO_2 and O_2 was used. In the earlier experiments with the cows only one or two such samples were taken during each 12 hours; and in a few cases the value used for carbon dioxide was that which was determined gravimetrically in a continous sample taken for use in determining the carbon balance, oxygen being determined gasometrically, as usual, but in continuous samples collected over water in an aspirator.

For the purposes of this comparison, in the experiments with cows the samples of the outcoming air were taken at half-hour intervals, exactly on the hour and half hour, during the last two consecutive 12-hour subperiods of a 48-hour calorimeter experiment.

In the experiments with steers the samples of the outcoming air were taken during every other subperiod, that is, during 12 hours of each experimental day. The samples were taken on the hour and the half hour except when the animal changed position shortly before the regular time for sampling, in which case an interval of at least 10 minutes was allowed to elapse before samples were taken.

Tables 2, 3, and 4 are presented as samples of the foundation data from which the heat production was computed, on planes of fasting, maintenance, and supermaintenance (production), respectively.

A study of these tables shows that the main influences which affected the composition of the outcoming air were the position of the animal, as to standing and lying, the plane of nutrition, and the time elasped since feeding. Also, since it would take from 25 to 40 minutes, according to the prevailing rate of ventilation, to pump through the calorimeter an amount of air equal to the capacity of the chamber, it is obvious that there was a very considerable lag—much more than 25 to 40 minutes—in the effect of the three above-mentioned influences on the composition of the outcoming air.

The effect of the position of the animal, as to standing and lying, is a complicated one, since it covers not only the characteristic rate of metabolism *in* the position, but also the exertion of *taking* the position; and the resultant from the combination of the approximately constant value for maintenance in a position with the diminishing effect of the effort of taking the position may hold over, on account of the instrumental lag, from an interval of time spent in one position through one or more subsequent intervals and positions.

TABLE 2.—*Percentage of carbon dioxide and oxygen in the outcoming air; experiment 235; periods 1a and 1b; steer 259; fasting*

| | Period 1a; subperiod 1 | | | | Period 1b; subperiod 1 | | |
Time of sampling	Position of animal	Carbon dioxide, by volume	Oxygen, by volume	Time of sampling	Position of animal	Carbon dioxide, by volume	Oxygen, by volume
		Per cent	*Per cent*			*Per cent*	*Per cent*
P. m. 6.10	Standing	0.349	20.510	A. m. 6.00	Lying	0.266	20.632
6.30	do	.383	20.486	6.30	do	.248	20.661
7.15	do	.360	20.504	7.00	do	.228	20.669
7.30	do	.355	20.519	8.00	Standing	.279	20.603
8.00	do	.352	20.505	8.30	do	.302	20.577
8.30	do	.335	20.527	9.00	Lying	.278	20.601
9.00	do	.332	20.545	9.30	do	.251	20.647
9.30	do	.338	20.528	10.35	Standing	.292	20.593
10.00	do	.321	20.549	11.00	do	.319	20.555
10.30	do	.337	20.531	11.37	Lying	.332	20.552
11.10	Lying	.320	20.553	Noon 12.00	do	.286	20.597
11.30	do	.314	20.580	P. m. 12.30	do	.256	20.640
Midnight 12.00	Standing	.338	20.535	1.00	do	.248	20.646
A. m. 12.32	Lying	.358	20.525	1.30	do	.263	20.633
1.00	do	.309	20.575	2.00	Standing	.336	20.545
1.30	do	.297	20.589	2.30	do	.350	20.516
2.00	do	.269	20.620	3.00	do	.344	20.532
2.30	do	.262	20.631	3.30	Lying	.305	20.567
3.00	do	.267	20.623	4.00	do	.277	20.610
3.40	Standing	.305	20.578	4.30	do	.272	20.612
4.10	Lying	.313	20.574	5.00	Standing	.357	20.470
4.30	do	.275	20.619	5.30	do	.417	20.473
5.30	do	.260	20.650				
6.00	do	.257	20.632				

TABLE 3.—*Percentage of carbon dioxide and oxygen in the outcoming air; experiment 221F; cow 887; period 2; maintenance*

| | Subperiod 3 | | | | Subperiod 4 | | |
Time of sampling	Position of animal	Carbon dioxide, by volume	Oxygen, by volume	Time of sampling	Position of animal	Carbon dioxide, by volume	Oxygen, by volume
		Per cent	*Per cent*			*Per cent*	*Per cent*
P. m. 6.00				A. m. 6.00	Standing	0.236	20.673
6.30	Standing	0.354	20.612	6.30	do	.317	20.601
7.00	do	.375	20.615	7.00	do	.357	20.578
7.30	do	.352	20.628	7.30	do	.321	20.618
8.00	do	.334	20.631	8.00	do	.318	20.634
8.30	do	.318	20.627	8.30	do	.322	20.590
9.00	do	.315	20.635	9.00	do	.314	20.598
9.30	Lying	.289	20.650	9.30	do	.288	20.616
10.00	do	.276	20.671	10.00	do	.295	20.548
10.30	do	.249	20.683	10.30	do	.284	20.651
11.00	do	.243	20.687	11.00	do	.286	20.620
11.30	do	.248	20.703	11.30	Lying	.272	20.638
Midnight 12.00	do	.238	20.712	Noon 12.00	do	.252	20.685
A. m. 12.30	do	.231	20.686	P. m. 12.30	do	.250	20.684
1.00	do	.258	20.671	1.00	do	.234	20.697
1.30	do	.254	20.659	1.30	do	.232	20.688
2.00	Standing	.260	20.630	2.00	do	.233	20.698
2.30	do	.260	20.638	2.30	do	.234	20.668
3.00	do	.256	20.640	3.00	do	.247	20.681
3.30	Lying	.268	20.652				
4.00	do	.240	20.666				
4.30	Standing	.232	20.678	4.30	Standing	.238	20.681
5.00	do	.245	20.656	5.00	do	.241	20.686
5.30	do	.245	20.654	5.30	do	.246	20.681
6.00	do	.236	20.673	6.00	do	.244	20.675

TABLE 4.—*Percentage of carbon dioxide and oxygen in the outcoming air; experiment 221F; cow 874; period 1; supermaintenance*

Time of sampling	Subperiod 3			Time of sampling	Subperiod 4		
	Position of animal	Carbon dioxide, by volume	Oxygen, by volume		Position of animal	Carbon dioxide by volume	Oxygen, by volume
		Per cent	*Per cent*			*Per cent*	*Per cent*
P. m. 6. 00	----------------	----------	----------	A. m. 6. 00	Standing_____	0. 331	20. 618
6. 30	Standing_____	0. 463	20. 518	6 30	_____do_____	. 442	20. 536
7. 00	Lying_____	. 506	20. 544	7. 00	_____do_____	. 490	20. 589
7. 30	_____do_____	. 464	20. 587	7. 30	_____do_____	. 462	20. 543
8. 00	Standing_____	. 431	20. 589	8. 00	Lying_____	. 463	20. 513
8. 30	Lying_____	. 438	20. 551	8. 30	_____do_____	. 402	20. 580
9. 00	_____do_____	. 390	20. 610	9. 00	_____do_____	. 375	20. 601
9. 30	_____do_____	. 358	20. 630	9. 30	Standing_____	. 413	20. 554
10. 00	Standing_____	. 386	20. 578	10. 00	Lying_____	. 426	20. 551
10. 30	Lying_____	. 406	20. 558	10. 30	_____do_____	. 379	20. 576
11. 00	_____do_____	. 357	20. 605	11. 00	_____do_____	. 353	20. 613
11. 30	_____do_____	. 335	20. 620	11. 30	Standing_____	. 401	20. 556
Midnight 12. 00	Standing_____	. 374	20. 580	Noon 12. 00	_____do_____	. 398	20. 559
A. m. 12. 30	Lying_____	. 363	20. 592	P. m. 12. 30	Lying_____	. 392	20. 569
1. 00	_____do_____	. 342	20. 631	1 00	_____do_____	. 335	20. 619
1. 30	Standing_____	. 367	20. 596	1. 30	_____do_____	. 327	20. 627
2. 00	Lying_____	. 376	20. 605	2. 00	Standing_____	. 375	20. 579
2. 30	_____do_____	. 318	20. 647	2. 30	Lying_____	. 346	20. 607
3. 00	_____do_____	. 310	20. 653	3. 00	_____do_____	. 325	20. 636
3. 30	Standing_____	. 329	20. 623	3. 30	_____do_____	. 316	20. 639
4. 00	Lying_____	. 330	20. 629	4. 00	Standing_____	. 350	20. 603
4. 30	_____do_____	. 313	20. 656	4. 30	_____do_____	. 344	20. 609
5. 00	Standing_____	. 349	20. 626	5. 00	_____do_____	. 357	20. 588
5. 30	_____do_____	. 335	20. 633	5. 30	_____do_____	. 341	20 603
6. 00	_____do_____	. 331	20. 618	6. 00	_____do_____	. 334	20. 598

We may conceive of the plane of nutrition as affecting the composition of the outcoming air in a rather definite manner, in view of the fact that the feed intake is normally constant throughout a metabolism experiment.

The effect of the time elapsed since feeding varies in another characteristic manner, rising and falling in a periodic variation coincident with the 12-hour interval between times of feeding.

In the feeding periods (Tables 3 and 4) there was invariably an increased percentage of carbon dioxide and a decreased percentage of oxygen in the air samples taken during the first hour or two after feeding (at 6 o'clock, morning and evening); and the fall in percentage of oxygen was generally less prominent than the rise in the percentage of carbon dioxide. This suggests that the increased outgo of carbon dioxide after feeding is not wholly due to muscular exertion, but is partly caused by increased emission of products of fermentation of the food, a process in which the oxygen involved may be at least in part, if not wholly, of intramolecular rather than atmospheric origin.

In the light of these observations it is obvious that the composition of the outcoming air, at any particular time, must be the resultant of such a complication of influences that no short-time period of observation can with propriety be assumed accurately to represent the whole day; and this situation would not be rendered satisfactory by taking samples of air only while the animal is in a standard position (either standing or lying), since this could, at best, obviate only one of a great complication of contributing influences.

The validity of this observation has been rendered especially clear through an effort of one of the writers to compute, from data such

as comprise Table 2 (fasting), an average or standard difference in heat production during standing and lying. So prominent were the effects on the outgo of CO_2, of the influences cited above, other than those related to the feed, that it was found, even with these factors eliminated, to be quite impossible, unless the animal remained in one position for an unusually long time, to obtain evidence of value on this factor; and since the half-hour air samples were found to be very often invalid for this purpose it is obvious that an average of analyses of a few half-hour samples can not be considered an entirely accurate basis for the computation of a respiratory quotient.

In computing the heat production of cattle by the respiratory-quotient method it is necessary to modify the usual procedure on account of the extensive fermentation which takes place in the ruminant alimentary tract.

Two methods of recognition of this fermentation factor have been proposed, one by A. C. Andersen (1) and one by Krogh and Schmit-Jensen (7).

In Andersen's method the computation of the nonprotein respiratory quotient is based on values for carbon dioxide produced and for oxygen consumed covering not only the metabolism of the animal but also the fermentation in the alimentary tract, and the complete oxidation of the methane produced. From the heat production as computed by the use of the respiratory quotient thus determined is subtracted the energy value of the methane itself; thus the heat of fermentation, the amount of which is unknown, is included, and the energy represented by the methane is excluded.

The following example of the use of this method is based on results of experiment 221F, cow 886, period 1.

Experiment with a milking cow:

CO_2 produced $= 2,670$ liters.
O_2 consumed $= 2,568$ liters.
CH_4 produced $= 254$ liters.
CO_2 produced if CH_4 is oxidized $= 254$ liters.
O_2 used if CH_4 is oxidized $= 508$ liters.
Energy of $CH_4 = 2,438$ Calories.
Nitrogen in urine $= 95.6$ gm.
CO_2 produced in protein metabolism $= 95.6 \times 4.75 = 454$ liters.
O_2 consumed in protein metabolism $= 95.6 \times 5.94 = 568$ liters.
Energy of protein $= 95.6 \times 26.51 = 2,534$ Calories.
CO_2 produced from nonprotein substances $= 2,670 + 254 - 454 = 2,470$ liters.
O_2 consumed by nonprotein substances $= 2,568 + 508 - 568 = 2,508$ liters.
Nonprotein R. Q. $= \dfrac{2,470}{2,508} = 0.985$.
Energy of nonprotein substances $= 2,508 \times [4.686 + 1.23(0.985 - 0.707)] = 12,610$ Calories.
Total heat production $= 12,610$ Calories $+ 2,534$ Calories for protein $- 2,438$ Calories in $CH_4 = 12,706$ Calories.
Constants employed:
Respiratory quotient for fat $= 0.707 = 4,686$ Calories per liter CO_2.
Respiratory quotient for carbohydrates $= 1.00 = 5.047$ Calories per liter CO_2.
From these values, for every 0.001 increase in respiratory quotient above 0.707 the heat per liter O_2 increases, above 4.686 Calories, by 0.00123 Calories.
Oxygen consumed per gram urinary $N = 5.94$ liters.
Calorific value per gram urinary $N = 26.51$ Calories.
Calorific value per liter O_2 consumed in protein metabolism $= 4.463$ Calories.
CO_2 produced per liter of O_2 consumed in protein metabolism $= 0.801$ liters.
CO_2 produced per gram of urinary nitrogen $= 4.75$ liters.

For places of publication and discussion of these factors see references (*1, 6, 8, 9, 11, 12*);[3] and for computing the heat production when the respiratory quotient exceeds 1.0 see references (*10, 13, 14*).

The special purpose of Andersen's (*1*) method may be considered to be to account for the heat of fermentation, which it does, along with all other factors of heat production. In effect, this method assumes that in the fermentation of carbohydrates in the alimentary tract either methane is the only incompletely oxidized product or, if there are other incompletely oxidized products of fermentation, their oxidation is completed after absorption from the alimentary tract, so that in any case after accounting for the incomplete oxidation of methane the relation between oxygen, carbon dioxide, and heat is the same as that prevailing in complete oxidation of carbohydrates.

Krogh's (*7*) method assumes that no atmospheric oxygen is consumed in the fermentation of carbohydrates in the alimentary tract; that is, that this process is strictly anaerobic, and, therefore, for the derivation of the nonprotein respiratory quotient an estimated amount of CO_2 due to fermentation is subtracted from the total CO_2 representing the metabolism of nonprotein substances, but no correction is made in the corresponding value for oxygen required. The CO_2 of fermentation is estimated from the methane production by the use of the factor 2.6, i. e., liters of methane $\times 2.6 =$ liters of CO_2. To the heat production as computed from this respiratory quotient and oxygen consumption is added the heat of fermentation, estimated to be 50 Calories per gram molecule of CH_4, or 2.25 Calories per liter of methane.

The application of this method is illustrated by Krogh and Schmit-Jensen (*7*) as follows:

The metabolism of nitrogen-free substances was found to require 1,451 liters of oxygen, and produced 1,583 liters CO_2 and 127 liters CH_4. The CO_2 of fermentation would be $2.6 \times 127 = 330$ liters, and the true respiratory quotient for the organism itself would be $\dfrac{1583 - 330}{1451} = 0.864$.

The energy metabolism is calculated from the oxygen intake by means of Zuntz's (*15*) formulas $E = O_2[4.686 + (R.\ Q. - 0.707)1.23]$.

In this case $E = 1{,}451[4.686 + (0.157 \times 1.23)] = 7{,}079$ Calories.

To obtain the total heat production it is necessary to add to 7,079 Calories the heat of fermentation, which, in this case, would be $127 \times 2.25 = 286$ Calories, and also the energy equivalent of the protein metabolized.

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[3] Certain constants which the writers have employed—5.94 for liters of oxygen per gram of urinary nitrogen and 4.463 for Calories per liter of oxygen consumed in protein metabolism—differ slightly from those used by some investigators. These differences are due to an arithmetical error in the publication of Loewy (*9, p. 279*), which was discovered by one of the writers, and to which it is here desired to call attention. In that publication the figure 5.91 is given instead of 5.94 as the result of the division $96.63 \div 16.28$; and dividing 26.51 by 5.94 gives 4.463 instead of 4.485, as in Loewy's paper.

TABLE 5.—*Average composition of ingoing and outcoming air [a] and total ventilation; experiments 221E, 221F, and 235; animals fasting*

Experiment No.	Animal	Period No.	Subperiod No.	Ingoing air			Outcoming air				Total ventilation	
				Carbon dioxide	Oxygen	Nitrogen	Carbon dioxide	Oxygen	Methane	Nitrogen	Ingoing	Outcoming
				Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Liters	Liters
221E	Cow 885	3	3	0.031	20.909	79.060	0.172	20.718	0.001	79.109	355,208	354,988
			4	.033	20.895	79.072	.178	20.701	.001	79.120	347,408	347,197
221F	Cow 874	3	3	.033	20.919	79.048	.179	20.730	.001	79.090	360,164	359,973
			4	.033	20.914	79.053	.180	20.715	.001	79.104	353,657	353,429
	Cow 887	3	3	b .331	20.915	79.054	.162	20.743	.000	79.095	361,918	361,731
			4	b .032	c20.915	79.053	.163	20.738	.000	79.099	360,442	360,232
235	Steer 259	1a	1	.039	20.925	79.036	.317	20.562	.002	79.119	234,409	234,163
		1b	1	.033	20.932	79.035	.295	20.588	.001	79.116	234,554	234,314
		1c	1	.038	20.922	79.040	.280	20.609	.001	79.110	231,151	230,947
		1d	1	.033	20.934	79.033	.267	20.626	.001	79.106	226,695	226,485
	Steer 260	1a	1	.032	20.929	79.039	.252	20.646	.001	79.101	232,473	232,291
		1b	1	.033	20.916	79.051	.235	20.660	.001	79.104	233,189	232,989
		1c	1	.033	20.929	79.038	.245	20.652	.000	79.103	227,547	227,360
		1d	1	.032	20.934	79.033	.228	20.679	.000	79.093	234,876	234,698

a All percentages are by volume, and all data in this table are on the dry basis.
b Gravimetric determination.
c Outside air sample lost; O_2 assumed to be the same as in subperiod 3.

TABLE 6.—*Average composition of ingoing and outcoming air [a] and total ventilation; experiment 221F; animals on feed*

Animal	Period No.	Subperiod No.	Ingoing air			Outcoming air				Total ventilation	
			Carbon dioxide	Oxygen	Nitrogen	Carbon dioxide	Oxygen	Methane	Nitrogen	Ingoing air	Outcoming air
			Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Liters	Liters
Cow 874	1	3 (night)	0.033	20.919	79.048	0.374	20.603	0.030	78.993	361,516	361,768
		4 (day)	b .032	20.910	79.058	.379	20.587	.030	79.004	358,576	358,821
	2	3 (night)	b .032	20.920	79.048	.281	20.675	.023	79.021	359,422	359,545
		4 (day)	.031	20.923	79.046	.284	20.669	.023	79.024	353,746	353,844
Cow 887	1	3 (night)	.033	20.907	79.060	.339	20.615	.029	79.017	351,775	351,966
		4 (day)	.031	c20.901	79.068	.370	20.578	.031	79.021	344,843	345,048
	2	3 (night)	b .033	20.880	79.087	.274	20.657	.021	79.048	360,927	361,105
		4 (day)	.032	20.898	79.070	.272	20.647	.020	79.061	361,083	361,124
Cow 886	1	3 (night)	.030	20.923	79.047	.413	20.541	.036	79.010	347,123	347,286
		4 (day)	b .031	20.917	79.052	.418	20.537	.037	79.008	346,114	346,307
	2	3 (night)	.030	20.905	79.065	.413	20.553	.038	78.996	354,659	354,969
		4 (day)	.031	c20.901	79.068	.426	20.535	.038	79.001	353,921	354,221
Steer 259	2	1 (day)	.033	20.928	79.039	.308	20.641	.019	79.032	318,692	318,720
		3 (day)	.032	20.930	79.038	.312	20.642	.020	79.026	318,163	318,211
		5 (day)	.033	20.930	79.037	.301	20.661	.020	79.018	323,070	323,148
	3	1 (day)	.035	20.916	79.049	.407	20.556	.030	79.007	321,308	321,479
		3 (day)	.037	20.914	79.049	.402	20.555	.030	79.013	319,540	319,686
		5 (day)	.032	20.916	79.052	.412	20.543	.030	79.015	320,168	320,318
Steer 260	2	1 (day)	.036	20.912	79.052	.300	20.657	.018	79.025	317,547	317,655
		3 (day)	.036	20.919	79.045	.299	20.651	.019	79.031	317,889	317,946
		5 (day)	.033	20.919	79.048	.301	20.650	.019	79.030	314,554	314,625
	3	1 (day)	.033	20.922	79.045	.387	20.580	.027	79.006	320,768	320,926
		3 (day)	.031	20.924	79.045	.390	20.576	.027	79.007	319,721	319,874
		5 (day)	.034	20.927	79.039	.386	20.580	.029	79.005	316,981	317,117

a All percentages are by volume and all data in this table are on the dry basis.
b Gravimetric determination.
c Determined in aspirator sample.

Tables 5 and 6 give the average analyses of the air, both ingoing and outcoming, and the total ventilation per subperiod of 12 hours.

We have no basis for judging the significance of the differences in the composition of the ingoing air; the composition is simply reported as determined.

In Table 5 the higher percentages of carbon dioxide and the lower percentages of oxygen, in the experiments with the steers, are due to the slower rate of ventilation which prevailed—this slower rate being as shown in the two columns giving the total incoming and outgoing air.

The very small amount of methane found in the outcoming air—a maximum of two parts per 100,000 in one subperiod with steer No. 259—shows that the animals were in an essentially true state of fast. Had there been feed residues in the alimentary tract, in any significant quantities, there would probably have been more considerable evidence of fermentation.

All figures for methane were computed to volume per cent, as reported, from continuous 12-hour gravimetric determinations.

The differences in analyses of the air in consecutive subperiods are to a considerable extent the consequences of variations in the electric power, as affecting the rate of ventilation.

A comparison of the analyses of the outcoming air in Table 5 with those in Table 6 shows the influence of feed on the respiratory exchange. Under the influence of feed more carbon dioxide and methane were produced, and more oxygen was used, than during fast.

From the differences in composition of the ingoing and the outcoming air, as exhibited in these tables, and the nitrogen of the urine, the respiratory quotients and the heat production were computed.

Both Andersen's (1) and Krogh's (7) methods require the same accounting for the protein metabolism, the basis for so doing being satisfactory in the experiments with steers on feed, since in these the urine was collected separate from the feces, and was composited during 18 days; while with the cows which received feed the conditions were less satisfactory, since with these the feces and urine were collected together, during only 10 days, and it was necessary to estimate the urinary nitrogen indirectly from the amount associated with the crude fiber as determined in the feces-and-urine mixture.

The estimation of the protein metabolism of the fasting steers was definitely unsatisfactory, in view of the irregularity of excretion of urine, and the fact that the excretion for only the last four days of fast could be used.

The estimation of the protein katabolism of the fasting cows was still less satisfactory since they were not catheterized, and since the amounts of urine and feces were based on a mechanical separation of these excreta for the two calorimeter days, after having been collected together.

DISCUSSION OF RESULTS

The respiratory quotients and dependent computations of the heat production are given for fasting cows and steers in Table 7, and for such animals on feed in Tables 8 and 9.

The nonprotein respiratory quotients for fasting steers (Table 7) were slightly above 0.7; thus, they were normal.

With the fasting cows the nonprotein respiratory quotients were appreciably below normal in four cases among six.

These low quotients might conceivably result from incomplete oxidation of fat, from synthesis of carbohydrate from fat or protein, or from inaccuracies in sampling or analytical work.

TABLE 7.—*Respiratory quotients and heat production of fasting animals*

Experiment, animal, period, and subperiod No.	Total CO₂ produced		Total O₂ consumed, intermittent sample	Respiratory quotients		Total heat production				
						Computed		Observed	Computed Observed	
	Intermittent sample	Continuous sample		Total	Nonprotein	Intermittent CO₂	Continuous CO₂		Intermittent CO₂	Continuous CO₂
Experiment 221E.									*Per cent*	*Per cent*
Cow 885—	*Liters*	*Liters*	*Liters*			*Calories*	*Calories*	*Calories*		
Period 3 {3 (night)	500.5	517.7	724.0	0.691	0.663	3,360.5	3,483.3	---------	------	-----
{4 (day)	503.4	510.7	717.6	.702	.676	3,330.9	3,382.0	---------	------	-----
Per day	------	------	------	------	------	6,691.4	6,865.3	6,575.6	101.8	104.4
Experiment 221F:										
Cow 874—										
Period 3 {3 (night)	525.5	527.4	720.3	.730	.713	3,347.4	3,361.5	---------	------	-----
{4 (day)	519.5	519.1	751.0	.692	.670	3,491.5	3,486.8	---------	------	-----
Per day	------	------	------	------	------	6,838.9	6,848.3	6,657.7	102.7	102.9
Cow 887—										
Period 3 {3 (night)	473.8	482.7	661.4	.716	.703	3,079.8	3,137.4	---------	------	-----
{4 (day)	471.9	479.8	681.4	.693	.676	3,173.7	3,227.6	---------	------	-----
Per day	------	------	------	------	------	6,253.5	6,365.0	6,227.9	100.4	102.2
Experiment 235:										
Steer 259—										
Period 1, subperiod 1 (night)	650.9	641.7	901.5	.722	.710	4,199.9	4,141.7	4,292.5	97.8	96.5
Period 2, subperiod 1 (day)	613.8	612.1	856.3	.717	.706	3,991.3	3,978.2	3,837.4	104.0	103.7
Period 3, subperiod 1 (night)	558.9	555.3	765.7	.730	.719	3,577.0	3,540.8	3,780.8	94.6	93.7
Period 4, subperiod 1 (day)	529.9	533.5	741.4	.715	.702	3,453.2	3,475.7	3,392.9	101.8	102.4
Steer 260—										
Period 1, subperiod 1 (night)	511.0	492.8	695.5	.735	.726	3,259.2	3,141.5	3,288.5	99.1	95.5
Period 2, subperiod 1 (day)	470.6	463.6	629.1	.748	.741	2,957.2	2,912.8	3,043.7	97.2	95.7
Period 3, subperiod 1 (night)	484.2	470.2	673.4	.718	.710	3,143.6	3,051.2	3,230.0	97.4	94.5
Period 4, subperiod 1 (day)	459.9	463.4	638.2	.721	.711	2,979.3	3,002.8	3,037.2	98.1	98.9

As bearing on this point, Carpenter (*2*) has shown that there is no appreciable fasting ketosis in cattle.

Also, in experiment 221F, with both cows 874, period 3, and 887, period 3, the respiratory quotients in one of the two consecutive subperiods is above 0.7, and in the other considerably below 0.7; further, in the cow experiments the pyrogallate solution used in the oxygen estimation was changed less frequently than in the steer experiments, and this may have resulted in low oxygen values in the outcoming air, high values for oxygen consumption, and low respiratory quotients. The probability, therefore, seems to be that these subnormal nonprotein respiratory quotients are due to imperfect technic.

In Table 8 are set forth the nonprotein respiratory quotients of the animals while on feed, these quotients being based on the volumetric analysis of the half-hour air samples, and being computed by the methods of Krogh (*7*) and of Andersen (*1*).

The quotients computed by the method of Andersen are all appreciably higher than those computed by the method of Krogh. The former are all close to 1.0, above or below, while the latter are in

general from one to two tenths less. In view of the funda-
mental difference in the procedures, which has been explained, the
quotients computed by the two methods have a somewhat different
significance, and therefore are not logically comparable as on the
same basis.

TABLE 8.—*Respiratory quotients and heat production of animals on feed, computed by Krogh's (7) and Andersen's (1) methods*

Experiment, animal, period, and subperiod No.	Total CO₂ produced, intermittent sample	Total O₂ consumed, intermittent sample	Respiratory quotients			Total heat production				
				Nonprotein		Computed		Observed	Computed / Observed	
			Total	According to Krogh	According to Andersen	According to Krogh	According to Andersen		According to Krogh	According to Andersen
Experiment 221F:	*Liters*	*Liters*				*Calories*	*Calories*	*Calories*	*Per cent*	*Per cent*
Cow 874—										
Period 1 {3 (night)___	1,233.7	1,090.4	1.131	0.896	1.093	5,473.0	5,455.5	-------	------	------
{4 (day)____	1,246.5	1,107.7	1.125	.897	1.089	5,556.0	5,539.6	-------	------	------
Per day_____	------	------	------	------	------	11,029.0	10,995.1	11,076.9	99.6	99.3
Period 2 {3 (night)___	893.5	855.2	1.045	.794	.998	4,213.5	4,230.8	-------	------	------
{4 (day)___	895.2	878.3	1.019	.774	.974	4,305.6	4,322.2	-------	------	------
Per day_____	------	------	------	------	------	8,519.1	8,553.0	9,125.8	93.4	93.7
Cow 887—										
Period 1 {3 (night)___	1,078.5	987.8	1.092	.827	1.041	4,912.4	4,917.0	-------	------	------
{4 (day)____	1,169.8	1,071.6	1.092	.840	1.040	5,340.6	5,344.2	-------	------	------
Per day_____	------	------	------	------	------	10,253.0	10,261.2	10,151.1	101.0	101.1
Period 2 {3 (night)___	870.3	768.1	1.133	.912	1.101	3,858.4	3,843.5	-------	------	------
{4 (day)___	866.8	897.8	.965	.736	.925	4,350.1	4,365.3	-------	------	------
Per day_____	------	------	------	------	------	8,208.5	8,208.8	8,348.8	98.3	98.3
Cow 886—										
Period 1 {3 (night)___	1,330.2	1,292.5	1.029	.773	.976	6,355.8	6,379.7	-------	-----	-----
{4 (day)____	1,340.3	1,275.6	1.051	.784	.994	6,298.8	6,326.0	-------	-----	-----
Per day_____	------	------	------	------	------	12,654.6	12,705.7	13,559.2	93.3	93.7
Period 2 {3 (night)___	1,359.6	1,184.7	1.148	.864	1.082	5,968.3	5,954.0	-------	------	------
{4 (day)____	1,399.3	1,233.7	1.134	.863	1.072	6,204.9	6,194.6	-------	------	------
Per day_____	------	------	------	------	------	12,173.2	12,148.6	12,483.4	97.5	97.3
Experiment 235:										
Steer 259—										
Period 2 {1 (day)____	876.5	908.8	.964	.787	.919	4,459.8	4,471.8	4,342.5	102.7	103.0
{3 (day)____	891.0	906.3	.983	.801	.935	4,466.0	4,479.3	4,346.1	102.8	103.1
{5 (day)____	866.1	853.0	1.015	.823	.962	4,231.7	4,243.9	4,299.4	98.4	98.7
Period 3 {1 (day)____	1,195.9	1,121.6	1.066	.846	1.001	5,620.0	5,640.0	5,771.7	97.4	97.7
{3 (day)____	1,166.9	1,117.2	1.044	.820	.980	5,567.5	5,587.5	5,645.5	98.6	99.0
{5 (day)____	1,217.2	1,163.5	1.046	.832	.984	5,806.4	5,826.3	5,711.0	101.7	102.0
Steer 260—										
Period 2 {1 (day)____	838.7	787.3	1.065	.886	1.015	3,952.1	3,958.4	4,069.6	97.1	97.3
{3 (day)____	836.3	840.4	.995	.814	.948	4,150.6	4,162.2	4,054.8	102.4	102.6
{5 (day)____	843.2	831.3	1.014	.830	.965	4,123.5	4,135.3	4,166.8	99.0	99.2
Period 3 {1 (day)____	1,136.1	1,064.4	1.067	.862	1.009	5,334.5	5,348.1	5,380.9	99.1	99.4
{3 (day)____	1,148.4	1,080.9	1.062	.859	1.005	5,413.6	5,427.7	5,236.6	103.4	103.6
{5 (day)____	1,116.3	1,071.9	1.041	.824	.981	5,333.7	5,352.6	5,245.4	101.7	102.0

The essential difference between the quotients computed by the
two methods is that the Andersen quotient covers the metabolism of
the animal and the oxidation of the methane, while the Krogh
quotient covers only the metabolism of the animal, and neither the
oxidation of the methane nor the CO₂ of fermentation. The different

use of the two quotients in the computation of the heat production is in harmony with this difference in significance, and yields results which are virtually identical. There is no point, therefore, in comparing these essentially different respiratory quotients.

TABLE 9.—*Heat production of animals on feed, computed by Anderson's (1) method, using CO_2 in continuous sample*

Experiment, animal, period, and subperiod Nos.	Total CO_2 produced	Non-protein respiratory quotient	Total heat production Computed	Computed / Observed
Experiment 221F:				
Cow 874—	*Liters*		*Calories*	*Per cent*
Period 1 {3 (night)	1,236.7	1.093	5,470.1	_____
{4 (day)	1,252.3	1.089	5,564.8	_____
Per day	_____	_____	11,034.9	99.6
Period 2 {3 (night)	967.2	.998	4,604.6	_____
{4 (day)	977.2	.974	4,743.5	_____
Per day	_____	_____	9,348.1	102.4
Cow 887—				
Period 1 {3 (night)	1,090.4	1.041	4,974.5	_____
{4 (day)	1,177.4	1.040	5,382.6	_____
Per day	_____	_____	10,357.1	102.0
Period 2 {3 (night)	884.6	1.101	3,908.1	_____
{4 (day)	883.4	.925	4,456.0	_____
Per day	_____	_____	8,364.1	100.2
Cow 886—				
Period 1 {3 (night)	1,458.6	.976	7,040.9	_____
{4 (day)	1,478.0	.994	7,023.0	_____
Per day	_____	_____	14,063.9	103.7
Period 2 {3 (night)	1,395.3	1.082	6,120.6	_____
{4 (day)	1,420.5	1.072	6,292.5	_____
Per day	_____	_____	12,413.1	99.4
Experiment 235:				
Steer 259—				
{1 (day)	885.0	.919	4,516.3	104.0
Period 2 {3 (day)	898.8	.935	4,519.5	104.0
{5 (day)	863.0	.962	4,229.4	98.4
{1 (day)	1,245.9	1.001	5,888.8	102.0
Period 3 {3 (day)	1,231.9	.980	5,917.9	104.8
{5 (day)	1,225.7	.984	5,867.5	102.7
Steer 260—				
{1 (day)	844.6	1.015	3,986.2	98.0
Period 2 {3 (day)	844.0	.948	4,201.6	103.6
{5 (day)	855.7	.965	4,199.4	100.8
{1 (day)	1,176.5	1.009	5,550.0	103.1
Period 3 {3 (day)	1,153.0	1.005	5,447.9	104.0
{5 (day)	1,139.8	.981	5,473.7	104.4

The heat production during fast was computed in two ways, as shown in Table 7—using the gravimetrically determined CO_2 from a continuous air sample and the volumetrically determined CO_2 from the intermittent half-hour samples, the intermittent O_2 being used, as determined, with the intermittent CO_2, but being corrected for use with the continuously determined CO_2 so as to give the same respiratory quotient as obtained from the intermittent CO_2 and O_2. In 6 out of 14 twelve-hour subperiods the intermittent CO_2 and O_2 gave the higher heat production; and the heat production as computed by either method was higher than the directly observed heat produc-

tion in 5 cases out of 11. Also, it is apparent that the mean deviation of the results based on the intermittent samples would be much less than that of results based on the continuous CO_2, with oxygen computed to give the same respiratory quotient as that derived from the intermittent CO_2 and O_2. This method of computation from the continuous CO_2 determination and an oxygen value computed to harmonize with the respiratory quotient determined from the intermittent air samples, seems to be of doubtful validity.

Table 8 compares the values for heat production as obtained by Krogh's (7) and Andersen's (1) methods. The practical identity of the results obtained by the two methods shows that even though they are based upon somewhat different considerations, and appear to be distinctly different, they are essentially the same; in other words, the factors used by Krogh for computing the CO_2 of fermentation and the heat of fermentation are such that the heat production computed by the two methods agrees almost exactly.

Table 9 exhibits the heat production of animals receiving feed, computed by the method of Andersen, from the continuous CO_2 determination, and the computed O_2 to correspond.

The correspondence between the computed and the directly observed heat production is good, but leaves improvement still to be desired, and such improvement seems perfectly practicable in the light of the obviously remediable imperfections of the technic followed in the respiratory quotient procedure. Among 18 comparisons all of the computed values were between 98 and 104.8 per cent of the directly observed values.

The desired improvements in the technic employed in the respiratory-quotient method are as follows: (1) A continuous and accurately representative sample of the outcoming air from the respiration chamber, for the determination of oxygen; (2) the number of oxygen estimations made with a given pyrogallate solution should be definitely regulated; (3) the method of accounting for the urinary outgo during fast should be improved either by catheterizing, if the subject is a female, or by observing the interval of time represented, and the weight of the separate portions as passed, if the subject is a male.

SUMMARY

The computation of the heat production of cattle by the respiratory-quotient method, as modified by either Andersen or Krogh on account of the extensive fermentation of carbohydrates in the ruminant alimentary tract, gives results which agree very well with direct heat measurements.

In spite of the fact that the procedure followed in the indirect method was imperfect in a number of details, in 18 comparisons with the direct method all of the computed values were between 98 and 104.8 per cent of the directly observed values.

Andersen's and Krogh's methods of computation of the heat production of cattle, while somewhat different in theory and while yielding respiratory quotients differing in magnitude and in significance, yield virtually identical values for heat production.

Eighteen determinations of the computed heat production according to Andersen, divided by the directly observed heat production, differed from a similar value involving Krogh's method by 0.1 to 0.4 per cent, the average being 0.24 per cent.

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In view of the variability in the composition of the outcoming air from the respiration chamber, as determined in samples taken at half-hour intervals, and as affected especially (1) by the position of the animal as to standing or lying, (2) by the activity of the animal, (3) by the time elapsed since change of position, and (4) by the time elapsed since feeding, no short-time period of observation can be assumed accurately to represent the whole day. The products of the extensive fermentation of carbohydrates constitute an important factor in this variation.

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SABAL CAUSIARUM (COOK) BECCARI: A NEW HOST OF THE COCONUT BUD-ROT FUNGUS[1]

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THE HOST

Sabal causiarum (Cook) Beccari is endemic in Porto Rico, where it is commonly known as the "hat" palm. It is most abundant in the southwestern section, where the leaves are used in the manufacture of hats, baskets, and other articles. The youngest leaf is cut as soon as it emerges from the inclosing sheath. At this stage the pinnae have not expanded and are of a cream color. They are bleached, split into narrow strips, and used for weaving, which is done entirely by hand. The young leaves are sold by the growers at 75 cents to $1.25 per dozen, and the palms are considered more valuable than coconut palms. Little effort has been made to grow the palm under grove conditions, and it is found largely in thickets or scattered through coconut groves. The palm is a very slow grower, and its slowness may be accentuated by the removal of its emerging leaves from the bud.

THE DISEASE

Early in 1926 the attention of the writer was called to a disease attacking hat palms growing a few miles south of Mayaguez. The disease was found to be a bud rot showing symptoms identical with those of coconut bud rot. The earliest indication of infection was the death of the young emerging leaf, followed by the death of a few of the youngest expanded leaves. This dead group eventually fell away, leaving the palm conspicuous by the absence of young leaves (figs. 1 and 2). The older leaves were not attacked, but died gradually, remaining attached to the trunk instead of falling away as do the leaves of bud-rot-infected coconut palms. The difference in symptoms is deemed of no significance and is due to the normal persistence of the petioles in Sabal.

The growing point of infected palms was reduced to a watery mass, emitting an odor which was indistinguishable from that associated with rotted coconut tissue. The bases of the young petioles were rotted off and the growing point with its leaf bases could easily be pulled from the inclosing sheath, leaving a cylindrical cavity about 6 inches in diameter and 18 inches in depth. Some of the older leaf sheaths bore brown decaying spots, similar to those in bud rot of coconut, previously recorded by Ashby (1),[2] Sundararaman (13), and the writer (15).

In 1923 a survey of the western coast of the island for coconut bud rot showed no cases of bud rot among either coconuts or hat palms south of Mayaguez. In 1926 a survey, conducted in cooperation with the Porto Rico Department of Agriculture and Labor, revealed 320 dying or recently dead coconut palms. The dying palms were infected by *Phytophthora palmivora* Butler. The survey was extended

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[1] Received for publication Dec. 11, 1926; issued June, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 887.

to include hat palms, and disclosed 23 dying and 189 recently
dead specimens.

In view of the facts (1) that the disease had become epiphytotic
among both coconut and hat palms at about the same time; (2) that
the hosts are closely related; and (3) that the symptoms of the disease

FIG. 1.—A healthy hat palm (*Sabal causiarum*). Note the erect young leaf in the center of the crown
and compare with Figure 2. The leaf blade was removed from the cut petioles for weaving

were identical on both palms, the coidentity of the causal organism
was regarded as highly probable. This opinion was further strength-
ened by the close and constant association of cases of bud rot of the
two hosts. In most cases a diseased coconut palm was found growing
within 75 feet of diseased hat palms, and in some instances the asso-

ciation was more intimate (fig. 3). No bud rot was found among either coconuts or Sabals growing about 2 miles north of the infected area.

THE ORGANISM

Microscopic examination of brown spots on leaf sheaths inclosed within the crown showed the presence of densely granular, nonseptate

FIG. 2.—*Sabal causiarum* attacked by bud rot. Note the absence of erect young leaves in the center of the crown

mycelium and chlamydospores (fig. 4). Isolations from such tissues resulted in pure cultures of a Phytophthora. Isolations from soft, decayed, growing-point tissues produced bacteria only. The same result had previously been obtained with coconut bud rot (15).

In pure cultures the Sabal strain behaves very similarly to the coconut strain. The two strains were grown in parallel cultures at room

temperature for comparison. The coconut strain referred to through-out this discussion is a subculture of the strain used by the writer (*15*) in 1924 to establish the causal organism of coconut bud rot in Porto Rico. Although the strain had been grown on culture media during two years, its reactions to the various media showed no perceptible differences from those observed when the strain was newly isolated.

Fig. 3.—Left, hat palm attacked by bud rot; right, coconut palm attacked by bud rot

On potato-dextrose agar plates both strains produced thin, ap-pressed, spreading, regular mycelia, which are radiate and homo-geneous from below. This radiate type of growth is to be distin-guished from the flocculent or lumpy, uneven type which is character-istically displayed by some Phytophthora strains. This radiate type is well described by Leonian's (*10*) term "smooth combed." After 72 hours the average diameter of the coconut strain was 29 mm.,

whereas that of the Sabal strain was 37 mm. In potato-dextrose agar both strains at two weeks showed profuse aerial mycelium, abundant chlamydospores, and fairly abundant conidia (sporangia). There was some germination by zoospores in the Sabal strain, but zoospores were not observed in the coconut strain.

On potato-agar plates the growth of both strains was thin, appressed, spreading, regular, and radiate to slightly granular.
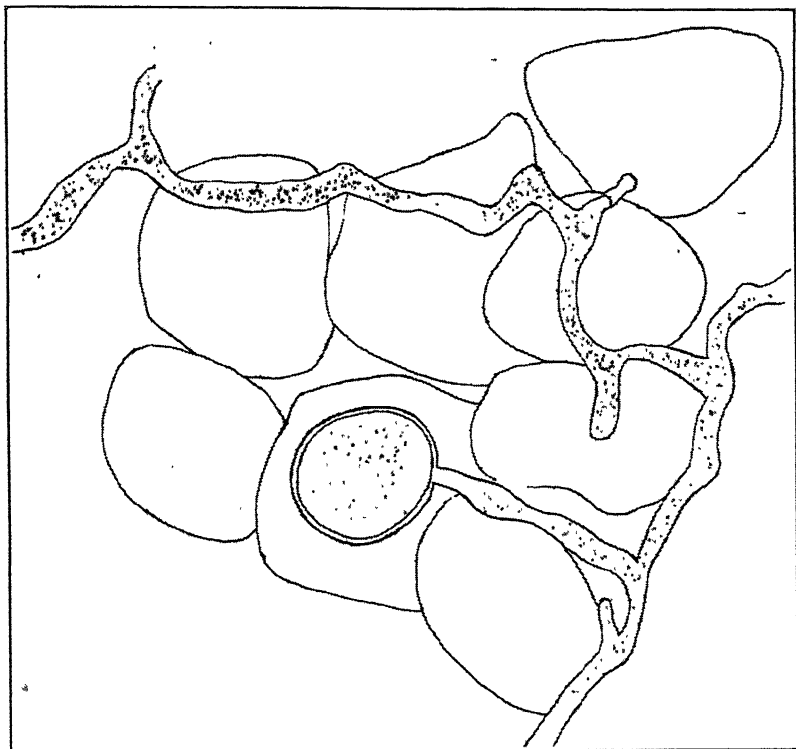
FIG. 4.—Hyphae and chlamydospore in a petiole of *Sabal causiarum* attacked by bud rot. × 644

After 72 hours the average diameter of the coconut strain was 47 mm.; that of the Sabal strain 38 mm.

On beef-dextrose agar plates both strains produced a meager, thin, appressed, slightly irregular, radiate growth. After 72 hours the coconut strain averaged 12 mm. in diameter, and the Sabal strain 11 mm.

On oatmeal-agar plates the colonies of both strains were very thin, appressed, spreading, regular, radiate. After 72 hours the coconut strain averaged 68 mm. in diameter, and the Sabal strain 61 mm. In oatmeal-agar tubes at two weeks both strains produced a medium quantity of aerial mycelium.

On bean-agar plates both strains produced thin, appressed, spreading, regular, radiate to flocculent mycelia. After 72 hours the coconut strain averaged 34 mm. in diameter, and the Sabal strain 29 mm. In tubes of bean agar at four months both strains

showed moderately profuse aerial growth, with abundant conidia and very few chlamydospores.

On steamed corn meal in flasks at two weeks both strains produced a frosty growth, with very little aerial mycelium and abundant conidia and chlamydospores. Some conidia were germinated by zoospore formation in the Sabal strain, but not in the coconut strain.

On steamed green bean pods the strains made very little aerial growth, but conidia and chlamydospores were abundant. Again the Sabal strain exhibited some zoospore germination, whereas the coconut strain did not.

The only perceptible difference between the strains was the slight tendency in the Sabal strain toward germination of the conidia by zoospores. The coconut strain rarely produces zoospores. This characteristic is not due to the length of time that it has been cultured, for similar results were recorded (*15*) when it was newly isolated.

For morphological comparison, 400 conidia from 13-day-old cornmeal cultures and 400 chlamydospores from 8 to 10 day old potato-dextrose agar cultures were measured. The measurements for the coconut strain were taken from a previous publication (*15*). Table 1 shows the great similarity existing between the two strains in the size of the spores.

TABLE 1.—*Dimensions of conidia and chlamydospores of Phytophthora palmivorao from coconut and Sabal causiarum bud rot*

Class (in microns)	Number of conidia in—				Number of chlamydospores in—	
	Coconut strain according to—		Sabal strain according to—		Coconut strain according to—	Sabal strain according to—
	Length	Diameter	Length	Diameter	Length	Diameter
15.5 to 17.49	0	0	0	1	0	1
17.5 to 19.49	0	2	0	3	0	1
19.5 to 21.49	0	2	0	8	0	10
21.5 to 23.49	0	3	0	17	9	11
23.5 to 25.49	0	2	0	15	18	13
25.5 to 27.49	2	18	3	27	30	18
27.5 to 29.49	1	56	5	111	56	32
29.5 to 31.49	2	94	6	92	54	82
31.5 to 33.49	2	138	6	82	81	73
33.5 to 35.49	2	56	5	20	42	56
35.5 to 37.49	1	18	7	14	41	43
37.5 to 39.49	1	11	3	10	29	52
39.5 to 41.49	4	0	8	0	17	28
41.5 to 43.49	9	0	11	0	13	19
43.5 to 45.49	18	0	9	0	6	5
45.5 to 47.49	22	0	21	0	2	4
47.5 to 49.49	44		20	0	2	2
49.5 to 51.49	36		32		0	
51.5 to 53.49	63		44		0	
53.5 to 55.49	32		27		0	
55.5 to 57.49	29		26			
57.5 to 59.49	30		29			
59.5 to 61.49	20		23			
61.5 to 63.49	16		29			
63.5 to 65.49	15		20			
65.5 to 67.49	10		20			
67.5 to 69.49	13		18			
69.5 to 71.49	5		11			
71.5 to 73.49	7		7			
73.5 to 75.49	4		3			
75.5 to 77.49	3		3			
77.5 to 79.49	6		2			
79.5 to 81.49	0		1			
81.5 to 83.49	0		1			
83.5 to 85.49	1					
85.5 to 87.49	0					
87.5 to 89.49	2					
89.5 to 91.49	0					
Total	400	400	400	400	400	400
Average	54.94	31.68	55.39	29.83	32.96	33.85

Four hundred conidia from a diseased Sabal petiole averaged 48.76 microns in length and 35.09 microns in diameter. They were somewhat shorter and thicker than those taken from corn meal. Four hundred chlamydospores from the same petiole averaged 32.60 microns in diameter.

Trial inoculations of various hosts with the two strains revealed further similarity. Wounded inoculated eggplant fruits showed slight infections after 48 hours with both strains. Neither strain caused any pathological symptoms in wounded eggplant seedlings 30 days after inoculation. The strains proved to be nonpathogenic to cacao fruits, and to cacao, *Erythrina poeppigiana*, breadfruit, and roselle seedlings. Both strains infected wounded papaya (*Carica papaya*) seedlings. The coconut strain caused the deaths of two of five inoculated seedlings in 7 days. After 60 days two of the remaining seedlings had dark lesions arising from the point of inoculation on the stem. The Sabal strain caused the deaths of three of five inoculated plants, one at 4 days and two at 6 days. The other two seedlings remained healthy. Another paper will show that few Phytophthora strains are pathogenic to papaya seedlings.

INOCULATIONS OF COCONUT PALMS

The foregoing comparisons of the coconut and Sabal strains of *Phytophthora palmivora* establish their identity as a single species; however, it is a well-known fact that physiologic races exist among the Phytophthora and cross-inoculation is the only completely reliable means of demonstrating the responsibility of any strain for disease in more than one host.

In April, 1926, twenty 15 to 25 year old coconut palms, growing in the station grounds remote from cases of bud rot, were inoculated by pouring a water suspension of the fungi among the emerging leaves. The palms were not wounded. Ten palms were inoculated with the Sabal strain and 10 with the coconut strain. The cultures for inoculation have been prepared by growing the fungi on steamed corn meal in liter flasks, and after 20 days adding sterile water to the flasks. On the twenty-first day each palm was inoculated with about 500 c. c. of the suspensions. Ten additional palms received a sterile suspension of corn meal and water.

On August 11, 115 days after inoculation, all palms were examined for evidences of infection. Of the 10 palms which had been inoculated with the coconut strain one proved to be a typical bud-rot case, the youngest leaf having died and the growing point having completely decayed. Eight palms showed spotting of the emerging leaves typical of this method of inoculation (*15*), and one palm showed no evidence of infection.

Of the 10 palms which had been inoculated with the Sabal strain, 3 developed into typical bud-rot cases. The young dead leaves were easily pulled from the crown and their bases and growing points were reduced to a watery, malodorous mass, which in every respect was identical with that produced by the coconut strain. The 7 remaining palms showed more or less severe spotting of the leaves. On the whole, the spotting caused by the Sabal strain was more severe than that caused by the coconut strain.

The 10 control palms showed no bud rot or leaf spotting.

The results of the inoculations are believed to furnish strong evidence in favor of the transmissibility of Sabal bud rot to coconuts, and to indicate the necessity for including *Sabal causiarum* with other host plants when a campaign is undertaken for the eradication of bud rot.

The coconut strain, which had been used in 1924 when four of eight inoculated palms developed bud rot in 108 days, showed an apparent slight loss of virulence. The season of 1926 has been fully as favorable for infection as was the summer of 1924 (*15*). Leonian (*10*) has shown that mutations occur in Phytophthora, and LaRue (*9*) has suggested that fungi may decrease in virulence in culture media, due to mutations giving rise to saprophytic strains while the parasitic strains perish. To determine whether the bud-rot Phytophthora really undergoes loss of virulence on culture media, inoculations are desirable with strains which have been isolated for a longer period than were the strains reported upon.

BUD ROTS OF OTHER PALMS

A careful search through the available literature for references to the occurrence of a bud rot on *Sabal causiarum* disclosed occasional notices of bud rots of other palms. The knowledge obtained is summarized here.

Butler (*3*) in 1907 described the occurrence in India of bud rots of the palmyra palm (*Borassus flabellifer* L.) and the betel nut or areca palm (*Areca catechu* L.). He later (*4*) determined that the bud rots of the palmyra and coconut palms were caused by the same organism, *Pythium* (*Phytophthora*) *palmivora* Butl.

Coleman (*5*) in 1910 found that occasionally the bud of the areca palm was affected by rot and that the nuts were the more important infection points. The fungus (*Phytophthora arecae* (Colem.) Pethyb.) is distinct from *P. palmivora* and is apparently nonpathogenic to the coconut bud.

Johnston (*8*) noted a disease of the crown of royal palms (*Roystonea regia* (H. B. K.) Cook) in Cuba in 1912. He stated, "In the royal palm the central leaves remained healthy longest, while the surrounding leaves gradually turned brown and fell off." He did not consider the disease a true bud rot.

In 1925 (*14*) a bud rot of royal palms was reported in Mauritius. It was thought to be of bacterial origin.

Van Hall (*6*) in 1921 recorded the presence in Java of a disease of the oil palm (*Elaeis guineensis* Jacq.) resembling bud rot. The cause was unknown. In 1922 (*7*) the oil palms in Sumatra were reported to be suffering from a crown disease which was thought to be due to physiological disturbances of unknown origin.

Sharples (*11*) in Malaya in 1922 investigated a kind of bud rot of young oil palms in which the disease began with the collapse of the third or fourth leaf from the center, followed by collapse of the outer leaves. The central leaves remained healthy. He suggested that the disease was an incipient form of bud rot and that the affected area was carried away from the healthy portion by the rapid growth of the central leaves. No organism was mentioned as a possible cause of the disease, and it was not observed to be fatal. In 1925 (*12*) he adopted the term "crown disease" for the trouble.

In 1924 Burger (*2*) recorded the presence in Florida of a disease similar to bud rot on *Cocos plumosa* Hook. He wrote: "The first symptom generally noted was the falling from the crown of the newest leaf before the blade unfolded. It gave off a putrid odor. The trees from which the buds have fallen linger for a season or two before dying, but eventually all diseased trees die." This description leaves little doubt that *Cocos plumosa* is susceptible to true bud rot, and the presence of *Phytophthora palmivora* on coconuts in Florida makes it very probable that *C. plumosa* is a host of *P. palmivora*.

SUMMARY

A bud rot of *Sabal causiarum* showing symptoms identical with those of coconut bud rot is recorded and described.

Bud rot appeared on both hosts at about the same time. Diseased palms of both genera were intimately associated.

A Phytophthora was isolated from Sabal and found to be indistinguishable morphologically and in culture reactions from a coconut-infecting strain of *P. palmivora*.

Both strains proved to be slightly pathogenic to wounded eggplant fruits, distinctly pathogenic to wounded papaya seedlings, and nonpathogenic to cacao fruits and cacao, roselle, *Erythrina poeppigiana*, breadfruit, and eggplant seedlings.

Ten unwounded coconut palms were inoculated with each strain. After 115 days inoculation with the coconut strain resulted in 1 typical bud-rot case, 8 cases with infected, spotted leaves, and 1 negative case; and inoculation with the Sabal strain resulted in 3 typical bud-rot cases, and 7 cases with infected leaves. Ten control palms showed no symptoms of infection.

The results establish *Sabal causiarum* as a host of *Phytophthora palmivora* and indicate the necessity for including it in eradication work for the control of coconut bud rot.

There is evidence of slight decreased virulence in the coconut strain after it has grown on culture media for two years.

Three hosts of the coconut bud-rot fungus—*Cocos nucifera, Borassus flabellifer*, and *Sabal causiarum*—have been definitely established. *C. plumosa* probably should be added to the list, although it has not been used as a host in cross-inoculation work.

Bud or crown diseases slightly resembling bud rot have been reported on *Roystonia regia* in Cuba and Mauritius and on *Elaeis guineensis* in Java, Sumatra, and Malaya, but evidence fails to show that either of these palms is susceptible to invasion by *Phytophthora palmivora*.

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THE DISCOLORATION OF CANNED CRANBERRIES [1]

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INTRODUCTION

Cranberry sauce preserved in tin cans sometimes becomes much discolored so that when the cans are opened the natural red color is found to have changed to a dirty brown, or black, which makes the sauce unappetizing in appearance. The causes of this discoloration have been under investigation at this experiment station.

A possible chemical cause of the change in color is the action of soluble aluminum, iron, or tin on the coloring matter of the cranberries, since compounds of these metals have long been used by dyers to modify their colors. In canning cranberries, the fruit is cooked in aluminum kettles and preserved in tin cans which are made of thin sheet iron coated with tin. The cans employed in the commercial preserving of cranberries are enameled; that is, the sheets of tin are coated with lacquer before cutting and bending them into cans.

PRELIMINARY EXPERIMENTS

Preliminary experiments with some cranberry sauce that had been prepared in glass were made by spreading the sauce in a glass dish and adding to the surface of the sauce a variety of soluble compounds of aluminum and of iron.

Aluminum compounds produced no perceptible color changes. Ferric chloride, ferric ammonium sulphate, and ferric ammonium citrate, when added to the sauce either in crystals or in drops, quickly darkened the red sauce in a widening area about the spots of application.

The best sauce obtained from tin cans showed a faint brown streak down the side where it was in contact with the seam of the can and also around the top. It was easy to observe the appearance of the sauce because it could usually be removed from the can as a cylinder of firm jelly. The most reasonable explanation of this discoloration appeared to be that in bending and cutting the sheet of tin, the lacquer and the tin coatings were slightly broken, thus exposing sufficient iron to the sauce to change its color.

Tannin had been noted as present in the cranberry in another investigation. The skins of the berries had been extracted with ordinary ether, which contained a little alcohol and water. The extract consisted of wax and resin, but darkened upon application of iron salts. When this reaction occurred, the extract was washed with hot water. The washings turned green on the addition of ferric

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No 9
May, 1, 1927
Key No. Mass.-18

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chloride and ferric acetate but did not indicate sufficient tannin present to produce the discoloration observed in the canned fruit.

It was considered advisable to isolate the pigment of the cranberry and to study its behavior toward the soluble compounds of aluminum, tin, and iron. Willstaetter and his students have studied the pigments occurring in many of the flowers and fruits, and their published papers have been compiled by Perkin and Everest.[2] Among the pigments described was the one occurring in the European cranberry, *Vaccinium vitis idaea.*[3] Willstaetter's method was followed in most of its details in extracting and purifying the pigment contained in our American cranberry, *Vaccinium macrocarpum.*

The coloring matter of the cranberry is confined almost wholly to the skin of the fruit, but when the skin is crushed or cooked some of the pigment diffuses throughout the pulp, coloring the mass.

EXPERIMENTAL PROCEDURE

Following Willstaetter's procedure, the cranberries were crushed and pressed to remove as much juice and pulp as possible. The moist residue, consisting mainly of skins, was placed in large glass flasks, and enough glacial acetic acid was added to cover the mass. The flasks were allowed to stand about one week, and their contents then filtered through glass wool. Owing to the presence of gelatinous matter it was almost impossible to filter the extract through paper or linen.

The residues were extracted a second and third time in a similar manner. The amount of pigment in the third extraction was slight, although the skins remained red in color. A further extraction with alcohol also failed to remove more than enough to color the solution.

The acid extract, which was brilliant red in color, was poured into two and one-half times its volume of ether, shaken repeatedly, and allowed to stand 24 to 48 hours. The pigment settled as a heavy, oily liquid from the first extraction but as a sticky, waxy mass from the third. The acid-ether liquid was slightly colored and was decanted carefully from the pigment. The ether and acetic acid were recovered by distillation and used for further extractions.

The pigment was purified after the method followed with the European cranberry, picric acid being employed first and finally hydrochloric acid to form crystalline compounds. The final crystals obtained with alcoholic hydrochloric acid were scalelike, lustrous, dark red, and almost black in some lights.

The crystals were readily soluble in methyl and ethyl alcohols but, unlike the pigment described by Willstaetter, they were only slightly soluble in water. It was also noticed that no successive crop of crystals from alcoholic solutions would redissolve completely in a fresh lot of alcohol, but would leave a slight precipitate, black in color and insoluble in methyl or ethyl alcohol, water, and acetic or hydrochloric acids. After the insoluble residue had been moistened with concentrated hydrochloric acid and ethyl alcohol had been added, a slight solubility colored the alcohol.

[2] PERKIN, A. G., and EVEREST, A. E. THE NATURAL ORGANIC COLOURING MATTERS. 655 p. London, New York [etc.], 1918.
[3] PERKIN, A. G., and EVEREST, A. E. Op. cit. p. 286.

Willstaetter obtained 1.6 gms. idaein chloride from 10.7 kgm. of cranberry skins, which would be equivalent to 40 kgm. of berries, calculated from other data given by him.

The writer used one-half barrel of Early Black cranberries, a highly colored American variety. Rejecting unsound fruit, approximately 22 kgm. of berries were used. The resultant masses of crystalline chloride of the pigment, when collected together, amounted to 2.1 gm. A half barrel of MacFarlin cranberries yielded much less in volume of crystals, but no weight records were taken. Early Black cranberries produce a much darker cooked product than other common varieties, due undoubtedly to the larger content of coloring matter in the berries.

A solution of 13 mg. of the hydrochloride dissolved in 15 c. c. of water and 3 drops of concentrated HCl, was prepared and used in the following tests. To 3 c. c. of color solution was added 3 c. c. of standard iron solution containing 0.3 mg. Fe. After several hours the color had become pale and a fine, granular, dark precipitate was noted. Minute crystals of ferric ammonium sulphate were added to 2 c. c. of the color solution, which was quickly decolorized with the formation of a fine, granular, dark precipitate. To a third portion of the color solution a small scale of ferric ammonium citrate was added, which slowly decolorized the solution with the formation of the dark precipitate. Aluminum salts showed no noticeable effects.

A methyl alcohol solution of the hydrochloride crystals was prepared, sufficient pigment being used to give a brilliant, clear, red color. Portions of about 3 c. c. each were used for tests with the different salts. Aluminum nitrate and ammonium aluminum sulphate caused little, if any, change in the solution. Stannous chloride produced a purplish tint. The iron salts darkened the solution and formed dark precipitates.

The contents of one can of cranberry sauce was much darkened, black particles were observed in the syrup, and the red color was nearly faded out. Several scratches which showed in the lacquer in the interior of the can appeared to be corroded. A weighed quantity of the darkened sauce (50 gm.) was dried and ashed. For comparison an equal amount of Early Black variety cranberry sauce, cooked and preserved in glass, was also ashed. The comparison of these two lots was made colorimetrically. By ordinary analytical methods cranberries normally contain no more than a trace of iron. The discolored product from the corroded can contained between six and seven times as much iron as the sauce from glass.

Some fresh acetic acid extract of the cranberry pigment was prepared from freshly crushed fruit. The pigment was precipitated by ether and redissolved in a little acetic acid. This acid solution was diluted with water to a clear, transparent, red tint and divided into two portions. To one portion a small coil of iron piano wire was added and to the other a small fragment of aluminum foil. The vessels containing these two portions, covered from dust, were allowed to stand for observation.

The solution with the aluminum foil was unaffected throughout the observation, which continued for two weeks.

The solution containing the iron wire began in the course of hours to produce a precipitate which steadily increased in amount, while the tint became progressively paler.

At the end of 72 hours the precipitate was collected by filtering through two filters of ash-free paper $4\frac{1}{2}$ cm. in diameter. The filters were repeatedly washed with hot water and were finally separated and dried. The inner filter contained all the precipitate. Each paper was incinerated in a clean porcelain crucible. The paper with the precipitate yielded an ash stained with iron, which on solution gave a bright color reaction for that metal. The other filter gave but a trace of ash and a very faint color reaction for iron. The evidence was positive that the precipitate contained iron and that the filter did not adsorb the iron from the solution.

SUMMARY

The discoloration of canned cranberry sauce is due to the formation of soluble iron from the inner surface of the can and to the reaction of the iron with the coloring matter and, to a less extent, with the tannin in the fruit.

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MOTTLE NECROSIS OF SWEET POTATOES [1]

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INTRODUCTION

In a brief preliminary article (*10*) [2] published in 1923, mottle necrosis was first described as a distinct disease of sweet potatoes (*Ipomoea batatas* Poir.). It was pointed out that specimens collected in various parts of the United States showed in cross section brown, irregularly formed spots. Attention was called to the fact that only occasionally was an organism obtained from plantings of necrotic tissue in agar plates. A few plantings gave bacteria, a species of Penicillium, and one or two species of Fusarium but mostly *Fusarium oxysporum* Schlecht. Inoculation experiments with *F. oxysporum* gave negative results, from which it was concluded either that this organism was not the causal agent or that the proper conditions for infection had not been obtained.

In the autumn of 1924 more intensive work was undertaken, and a microscopic examination of decayed potatoes revealed the presence of a phycomycete which had not been isolated by any of the methods employed. A modification of the methods resulted in the isolation of this fungus (*7*), which was proved by numerous inoculation experiments to be the cause of the disease. Detailed investigations have since been made, the results of which are contained in this article.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Mottle necrosis, collected for the first time in 1917 on sweet potatoes of the Yellow Jersey variety at the Arlington Experiment Farm, Rosslyn, Va., has increased in importance each succeeding year. In 1918 Lauritzen collected material in eastern North Carolina on the same variety. Mottle necrosis was prevalent in New Jersey in the autumn of 1925, and farmers told the senior writer that it had occurred off and on in the State for 20 years or more.

In 1890 Halsted (*5*) described a disease of sweet potatoes in New Jersey which he called white rot. His description of white rot, probable method of infection, and other data concerning the fungus associated with it, suggest its probable identity with mottle necrosis. Halsted apparently did not study the disease in the field but called attention to the fact that, in view of the depressions about the small rootlets, infection probably occurred through them. He also pointed out that a badly decayed sweet potato might show very little evidence of decay at the surface, which is sometimes true of mottle necrosis; also that when superficially conspicuous the spots might unite and become somewhat sunken. A fungus with thick-walled

spherical bodies, which might conceivably be an oömycete, was found associated with the disease. If one assumes from the description given by Halsted that white rot is the same as mottle necrosis, the disease has been known to science for at least 37 years.

No survey of the distribution of mottle necrosis has been attempted. Specimens have been collected in or received from Virginia, Maryland, Delaware, New Jersey, Mississippi, North Carolina, and South Carolina. Thus far it has been found mostly in States where the more susceptible varieties of sweet potatoes (Yellow Jersey and Big Stem Jersey) are grown. Only a few specimens of the disease have been received from Mississippi and no report of its prevalence there has ever been made. Whether it is widespread in the Southern States, or ever will become so, remains to be determined. Although one of the most susceptible varieties (Triumph) is grown commercially in Alabama, mottle necrosis has not been observed or reported from that State. Judging from the knowledge now available of the environmental requirements of the causal organisms, it would be surprising if it ever became very destructive in the South. There are two plausible theories to account for this: First, the varieties of sweet potato grown in the South are generally more or less resistant under field conditions, with the exception of the Triumph; and, second, results to be presented later indicate that the prevailing high temperature of the South might be a limiting factor to any general destruction of sweet potatoes in the field.

ECONOMIC IMPORTANCE

No reports of a widespread destructiveness of this disease throughout the sweet-potato-growing districts have been recorded. Losses of from 10 to 40 per cent have occurred in New Jersey and during different seasons in certain varieties grown near Rosslyn, Va. Judging from these casualties, mottle necrosis is potentially a serious disease, and, in seasons when optimum conditions for infection occur, reports of extensive losses would not be surprising.

SYMPTOMS

It is practically impossible to describe mottle necrosis in a way sufficiently accurate to differentiate it in all cases from rots caused by other fungi. If material for examination could be obtained at just the proper time and under suitable conditions it could be identified macroscopically with a reasonable degree of accuracy. On the other hand, if one is in doubt as to the identity of the disease, a microscopic examination of the decayed tissue will show the presence of mycelial threads of a phycomycete in and about the cells. Even after the organism is dead and can no longer be isolated, the large, nonseptate, vacuolated mycelial threads can be clearly distinguished. Mottle necrosis is primarily a field disease, and when it occurs it is found at digging time, being in this respect different from any of the other diseases of the roots with the possible exception of soft rot, caused by *Rhizopus nigricans* Ehrb., which may occasionally be found in the field.

Superficially, mottle necrosis may be quite inconspicuous. The surface lesions vary from small, dirty brown, somewhat sunken spots at the base of the small rootlets to conspicuous sunken spots of various

sizes and shapes.   The presence of a small lesion on the surface does not necessarily indicate a small amount of decay within.   Frequently such sweet potatoes may be almost entirely decayed or they may be decayed only in localized areas.   On the other hand, the surface may

FIG. 1.—Mottle necrosis of sweet potatoes. External symptoms on the Yellow Jersey variety.
(About three-fifths natural size)

be more or less covered with brown, sunken spots (fig. 1), and when the potatoes are cut open it may be found that very little internal decay has actually taken place.

FIG. 2.—Mottle necrosis of sweet potatoes. A and B, transverse and longitudinal sections, respectively, of sweet potato, showing cheesy type of decay produced by artificial inoculation. C, transverse section of sweet potato, showing natural infection of the band type of decay. D and E, transverse and longitudinal sections, respectively, showing natural infection of marble type of decay. (All about two-thirds natural size)

When a diseased root is cut in two longitudinally (fig. 2, B and E) or transversely (fig. 2, A, C, and D), any one of three types of decay may be identified, depending to a considerable extent upon the local weather conditions which prevailed at the time decay was in progress. For the sake of classification these have been designated the marble type (fig. 2, D and E), the cheesy type (fig. 2, A and B), and the band type (fig. 2, C), the last being the least common. The band of necrotic tissue characteristic of the band type is very shallow, seldom extending beneath the fibrovascular ring, although penetration at some local point may occasionally occur. The sweet potato is firm and the chocolate-brown color of the surface is the only evidence of decay. The surface is not usually sunken, although after drying for some time (as, for example, curing in storage) some shrinking may occur. A better idea of this type of decay can be gained from a cross section through the root, which will show a thin superficial layer of necrotic tissue about 1 to 3 mm. thick.

There are well-marked characteristics that differentiate the typical marble and cheesy types of decay, both of which are encountered in the field, and, it was believed at the outset, the different symptoms were produced by different causal organisms. It was shown later that either type could be produced by more than one organism, the kind of decay depending largely upon atmospheric and soil conditions.

The marble type, shown to the best advantage by cutting a section through the root, is more prevalent in the late summer and early fall. The irregularly shaped islands of necrotic tissue of various sizes scattered more or less indiscriminately through the root are characteristic of the marble type. The organism ramifies from the point of infection in various directions, forming chambers or pockets of decayed tissue which might be assumed from a cursory examination to be disconnected. If, however, the different pockets and chambers are followed through their various ramifications, they will be found to be connected or to have a common point of origin. The necrotic tissue is dry, crumbly, and from a dark gray to a chocolate brown in color.

In a typical example of the cheesy type as found in the field or produced by artificial inoculation, the tissue is of about the consistency of soft cheese and of a grayish color. Instead of the fungus ramifying in many directions and forming almost isolated pockets, large chambers with comparatively smooth walls are produced, closely resembling those caused by Rhizopus, but less watery.

In attempting to identify this disease from a macroscopic examination, account must be taken of the conditions under which the sweet potatoes have been held. Experiments to be discussed later have shown that the color of the decayed tissue is correlated rather closely with the loss of water. If the water escapes freely the tissue will turn brown rapidly. If, on the other hand, there is very little loss of water, the color of the rotted tissue will remain practically the same as that of the healthy.

THE CAUSAL FUNGI

From the numerous inoculation experiments which have been conducted with the various strains of Pythium isolated from sweet potatoes affected with mottle necrosis, two species, identified by Drechsler, have proved to be pathogenic. The first of these, isolated

but a few times, is *Pythium scleroteichum* Drechsler (in manuscript), and is a new species to be described later by Charles Drechsler, probably in the JOURNAL OF AGRICULTURAL RESEARCH. The other species of proved pathogenicity is *P. ultimum* Trow, which was described (*17*) in 1901 as a saprophyte and is the species most frequently encountered in mottle necrosis. The present writers (*9*) have shown that this species causes a leak in artifically inoculated snap beans similar to that caused by *P. aphanidermatum* (Edson) Fitzpatrick.

The last-named organism, isolated from snap beans, has been employed in some of the investigations herein recorded and has proved to be a rapid grower and a virulent parasite on sweet potatoes, producing typical mottle necrosis.

The taxonomy of the fungi causing mottle necrosis has not been studied, the writers prefering to leave that phase of the subject to Drechsler, who is monographing the genus.

PATHOGENICITY

METHOD OF INOCULATION

Several methods of inoculation to test the pathogenicity of the organisms in question were tried before a convenient and successful one was found. A method previously used (*11*), in which the organism was grown in sweet-potato decoction for the study of the parasitism of different species of Rhizopus, was tried and found to be unsuited for Pythium. Liquid media did not, in general, give satisfactory growth, so a solid medium was eventually employed. From the different methods tried the one selected as being the most satisfactory may be described briefly as follows:

A planting is made of the organism to be used on 10 c. c. of corn-meal agar in a 90 mm. Petri dish. At the end of about two days a copious growth covers the entire plate at a temperature of approximately 25° C. Inoculations are made by what may properly be called a "well" method. A hole about 1.5 cm. deep is made into the sweet potato with a cork borer, and into the hole is placed approximately 5 sq. cm. of the corn-meal agar on which the organism is growing and the plug replaced after removing about 0.5 cm. of the lower end to allow for the inoculum. The plug forms a reasonably close seal over the agar. To prevent too rapid drying out of the culture, the plug is smeared with a thin coating of vaseline, which practically cuts off the escape of moisture. The sweet potatoes thus inoculated are placed in baskets or any covenient receptacle and held at a suitable temperature for growth. A temperature of 25° has been mostly employed for this work, although it has been shown that the optimum is somewhat lower.

INFECTION TESTS

Numerous inoculation experiments and reisolations have been made in testing the parasitism of the various species of Pythium and the susceptibility of the different varieties of sweet potatoes, all of which will not be detailed, especially those of a preliminary character. In view of the extreme susceptibility of the Yellow Jersey variety and its general distribution in the localities where mottle necrosis occurs, it has been employed for much of the inoculation work.

Literally hundreds of isolations of Pythium in pure culture have been made, many of which have been used in inoculation experiment tests, while others have never been identified. At the outset a large number of inoculations were made for the purpose of obtaining a suitable method. None of these preliminary results are included in the data shown in the tables. It is believed, however, that a sufficient number of positive results have been obtained to justify the conclusion that several species of Pythium are capable of causing a decay of sweet potatoes. All organisms except those from beans and sweet potatoes were obtained from Drechsler.

TABLE 1.—*Inoculation of different varieties of sweet potatoes with Pythium ultimum from various hosts and P. scleroteichum from sweet potatoes*

Variety inoculated	Source of organism	Pythium ultimum			Pythium scleroteichum		
		Number of potatoes inoculated	Number diseased	Percentage diseased	Number of potatoes inoculated	Number diseased	Percentage diseased
Big Stem Jersey	Sweet potato	14	9	64.3	13	7	53.8
Do	Watermelon	7	7	100.0			
Do	Pea	6	6	100.0			
Do	Pelargonium	7	7	100.0			
Nancy Hall	Sweet potato	6	5	83.3	6	3	50.0
Porto Rico	do	16	10	62.5	6	2	33.3
Triumph	do	6	6	100.0	13	13	100.0
Yellow Jersey	do	154	150	97.4	29	16	55.1
Do	Cabbage	6	6	100.0			
Do	Potato	6	6	100.0			

The data given in Table 1 show that *Pythium ultimum* and *P. scleroteichum* are parasitic on a few varieties of sweet potatoes. The former organism is commonly found on the rootlets and in potatoes affected with mottle necrosis, but the latter is only occasionally isolated. The results of the inoculations show that *P. scleroteichum* is capable of infecting several varieties of sweet potatoes, although the percentage of infections is, on the average, lower than that for either *P. ultimum* or *P. aphanidermatum*, as will be shown later. Although no difference could be detected in the morphology of the organisms from the different sources, nothing was known with respect to their comparative capacities for parasitism. The importance of this information will be appreciated if it is remembered that some of these crops (cabbage, watermelon, potato, and pea) may be, and frequently are, used in rotation with sweet potatoes. The results of the inoculations of several varieties of sweet potatoes with *P. ultimum* from five different sources in comparison with the same organism from sweet potatoes show that there is no varietal difference in the parasitism of the various strains.

The percentage of infection by *Pythium scleroteichum*, as shown in Table 1, is considerably less than that by *P. ultimum* and probably represents fairly accurately the comparative parasitic capacities of the two organisms. *P. ultimum* is a much more vigorous grower in pure culture than *P. scleroteichum* and causes on the whole a more rapid decay. At one time it was suspected that the latter produces only the marble type and the former the cheesy type, but such is not the case, since either form of decay may be produced by either species provided the environmental conditions are favorable.

VARIETY TEST

Investigations by Braun (*1*, *2*), Drechsler (*3*, *4*), Hawkins (*12*), Jones (*14*), and others (*9, 15, 16*) have shown that Pythiums occur on a variety of hosts widely separated in relationship; so to find them on the roots of sweet potatoes occasioned no surprise. In 1925 Harter (*6*) pointed out that the root tips of sweet potatoes in the hotbed were conspicuously injured by a species of Pythium of the debaryanum type. Out of the 21 varieties examined all were injured, but some to a greater extent than others. In view of these results, it is likely that all varieties of sweet potatoes are potential hosts to species of Pythium.

Field observation among 21 varieties of sweet potatoes grown near Rosslyn, Va., showed that the Triumph, Yellow Jersey, and Big Stem Jersey are the most susceptible varieties, although infections of some of the others were observed occasionally. Experiments have shown that the species of Pythium causing mottle necrosis is identical with the one almost uniformly isolated from the root tips of plants in the hotbed. Inasmuch as mottle necrosis is uncommon on most of the varieties of sweet potatoes under field conditions, a test of the susceptibility of 21 varieties, many of which are grown extensively commercially, was made with *Pythium ultimum* and *P. aphanidermatum*. These inoculations were made with sweet potatoes which had been held in storage for several weeks, previous investigation having shown that they may be successfully infected at any age.

TABLE 2.—*Results of inoculation of different varieties of sweet potatoes with Pythium ultimum and P. aphanidermatum*

Variety inoculated	Pythium ultimum			Pythium aphanidermatum		
	Number of potatoes inoculated	Number diseased	Percentage diseased	Number of potatoes inoculated	Number diseased	Percentage diseased
Big Stem Jersey	14	13	92.8	6	6	100.0
Creola	6	6	100.0	6	6	100.0
Dahomey	6	6	100.0	6	6	100.0
Dooley	6	5	83.3	6	2	33.3
General Grant Vineless	6	6	100.0	6	6	100.0
Georgia	9	9	100.0	8	8	100.0
Gold Skin	6	6	100.0	6	6	100.0
Haiti	6	6	100.0	6	6	100.0
Key West	6	6	100 0	6	6	100.0
Nancy Hall	6	5	83.3	6	6	100.0
Pierson	6	6	100.0	6	6	100.0
Porto Rico	16	9	56.3	16	10	62.5
Pumpkin	6	4	66.7	6	6	100.0
Red Brazil	6	6	100.0	6	6	100.0
Red Jersey	6	6	100.0	6	6	100.0
Southern Queen	6	6	100.0	6	6	100.0
Triumph	6	6	100.0	6	6	100.0
White Yam	6	6	100 0	6	6	100.0
Yellow Belmont	6	6	100.0	6	6	100.0
Yellow Strasburg	6	6	100.0	6	6	100.0
Yellow Jersey	[a] 182	161	88.5	6	6	100.0

a Includes potatoes used in other experiments.

Table 2 shows, first, that with a few exceptions all the varieties tried were 100 per cent infected with *Pythium ultimum* and *P. aphani-*

dermatum; second, that some of the varieties never found infected under field conditions were 100 per cent infected artificially; and third, that some of the susceptible varieties (Yellow Jersey, Big Stem Jersey) under field conditions were not all infected. This is not to be interpreted, however, as indicative of any resistance on the part of such varieties, but rather of their having escaped infection. More varieties were 100 per cent infected with *P. aphanidermatum,* the species from beans, than with *P. ultimum,* the sweet-potato form.

RESULTS WITH DIFFERENT SPECIES OF PYTHIUM

The data so far recorded are the results of inoculations with only three species of Pythium, of which two (*P. ultimum* and *P. scleroteichum*) were isolated from sweet potatoes and the other (*P. aphanidermatum*) from beans. Other species of Pythium occur on the roots or other parts of plants some of which may be and frequently are used in crop rotation with sweet potatoes. Inasmuch as the sweet potato might be exposed to possible infection by such species, several of them were obtained from Drechsler and a few sweet potatoes (Yellow Jersey) were inoculated by the usual method. These organisms were compared in parallel experiments with those obtained by the writers from sweet potatoes and beans.

Out of the many isolations made from the rootlets and enlarged roots of sweet potatoes, occasionally an undetermined species of Phytophthora was obtained. While it was suspected that this organism had no causal relationship to mottle necrosis, a number of inoculations were made, the results of which will be set alongside those with the different species of Pythium.

TABLE 3.—*Results of inoculating sweet potatoes (Yellow Jersey) with different species of Pythium and with Phytophthora sp.*

Species	Host	Number of potatoes inoculated	Number diseased	Percentage diseased
Pythium:				
Adhaerens [a]	Cucumber	12	0	0.0
Aphanidermatum	Bean	6	6	100.0
Artotrogus	Pea	32	0	0.0
Complectens	Pelargonium	12	0	0.0
Species 5825	Pear	12	1	8.3
Debaryanum	Sweet potato	24	19	79.1
Megalacanthum	Lettuce	12	0	0 0
Splendens	Pelargonium	12	11	91.7
Ultimum	Sweet potato	82	80	97.6
Scleroteichum	____do____	29	16	55.1
Phytophthora sp	____do____	48	0	0.0

[a] This is a new species to be described by Drechsler later, probably in the Journal of Agricultural Research.

The data in Table 3 show that neither mottle necrosis nor any other type of disease of sweet potatoes is caused by the species of Phytophthora tested. It is probable that this organism, like many others, finds its way into the roots as a secondary invader and can in no way be assumed to bear any causal relationship. With respect to the other organisms, the results indicate that five species of Pythium may cause decay of sweet potatoes by the methods of inoculation employed. The question naturally arises, and especially in the case

of some of the Pythium species, as to what actually constitutes,'' infection. The results in the case of some of the species are so striking as to leave no doubt. For example, *P. ultimum*, *P. debarya-num* Hesse, and *P. aphanidermatum* produce a characteristic and abundant rot. Contrasted with these unquestionably parasitic species are those which cause absolutely no decay and would un-hesitatingly be classed as nonparasitic. There is in Table 3 a third group, consisting of two species (*P. splendens* Braun and *P.* 5825), which have been classed as parasites, about which there is some question. The method employed in the inoculation was the usual one in which a plug was removed from the sweet potatoes by means of a cork borer, the inoculum put in the hole, and the plug replaced. Naturally, some of the cells adjacent to the cut made by the cork borer would be mutilated and therefore would be unable to offer the same resistance to invasion by fungi as the unwounded ones. Both *P. splendens* and *P.* 5825 caused the formation of a narrow fringe of necrotic tissue adjacent to the cut surface in contact with the inocu-lum. In no case was a measurable amount of dead tissue produced. There was more, however, than occurred in the controls receiving similar mechanical injury but no inoculum. For this reason the writers have placed these two species in the parasitic class, though it must be kept in mind that they are extremely weak parasites on sweet potatoes.

SUMMARY OF INOCULATION EXPERIMENTS

Out of hundreds of isolations from decayed rootlets and mottle-necrosis sweet potatoes, two species of Pythium have predominated, *P. ultimum* and *P. scleroteichum*. Both of these will produce typical symptoms of mottle necrosis of the marble or the cheesy type. A species of Phytophthora was isolated several times, but in no case did it cause a decay as a result of inoculation experiments. Another species of Pythium, *P. aphanidermatum*, isolated from beans, was found to be extremely parasitic on sweet potatoes. This species, together with *P. ultimum* and *P. scleroteichum* and the very weakly parasitic forms, *P.* 5825 and *P. splendens*, must be regarded as the only species parasitic on sweet potatoes known at the present time. Under field conditions only three varieties of sweet potatoes (Tri-umph, Yellow Jersey, and Big Stem Jersey) have shown any marked susceptibility to mottle necrosis. However, inoculation experi-ments of sweet potatoes from storage have shown that all the 21 varieties tried can be successfully infected. In view of this fact, most if not all of the commercial varieties may be regarded as poten-tially susceptible to mottle necrosis.

DISSEMINATION

So far as known, there is nothing distinctive with respect to dis-semination of the causal organism. Pythiums are known to be widely distributed in the soil and on plant parts, so their presence might be expected in almost any situation. A rootlet rot caused by Pythium occurs in the hotbed on all varieties, and, although it is present every year, it is worse some seasons than others, the prev-alence of the disease probably depending upon environmental con-

ditions. Rootlet rot occurs in beds prepared by the use of sand and also in soils containing a considerable quantity of organic matter in the form of stable manure. The disease, therefore, probably is carried to the field on the plants. This, however, does not necessarily mean that because plants with diseased rootlets are planted mottle necrosis will occur in the field.

If conditions are favorable, the repeated use of the same hotbed soil appears to increase the amount of rootlet rot; also repeated planting in the same ground results in increased mottle necrosis in the field.

A certain relationship seems to exist between the soil type and mottle necrosis, although this phase of the problem has not been thoroughly investigated. As proof of this it may be pointed out that when the Yellow Jersey variety of sweet potato is planted across a field containing a loam, a sandy loam, and a very light sandy soil, the disease, while occurring on all three, is more prevalent on the sandy loam, showing that very heavy and very light soils are more or less unfavorable to the disease. To what extent soil moisture is a factor in infection has not been determined, although it is observed that during dry seasons infections are deeper in the ground than in wet ones, the moister earth seemingly contributing to infection. It is likely that an amount of soil moisture sufficient to permit zoöspore migration and mycelial development would somewhat facilitate infection.

METHOD OF INFECTION

Inasmuch as infection takes place underground, it is impossible to acquire any exact information as to just how it is accomplished. *Pythium ultimum* or *P. scleroteichum* has been isolated from the small rootlets on the fleshy roots connecting with cavities or chambers of necrotic tissue within the potato. Reasoning from these observations and from the results of the isolation of parasitic species of Pythium from the short dead rootlets and from the cavities of necrotic tissue connecting with them, there would seem to be little doubt that the small rootlets are the primary infection courts.

EFFECT OF THE FUNGUS ON THE HOST

A sweet potato freshly decayed with Pythium under favorable environmental conditions is found, on cutting in two, to be spongy or of about the consistency of a soft cheese. In the early stages of decay the color is very little changed, but upon exposure to the air it darkens, simulating old decayed tissue. The sponginess of the decayed tissue is doubtless due to the loss of turgidity caused by the death of the cells accompanied or followed by the dissolution of the middle lamellae. No studies have been made of the enzymes secreted by Pythium, but reasoning from what is known of the enzymic activities of Rhizopus spp. (8) causing soft rot of sweet potatoes, it is probably safe to assume that an enzyme, pectinase, is secreted which dissolves the middle lamellae in advance of the growing hyphae. Some justification for this assumption may be claimed in view of the fact that, as in the case of Rhizopus, there is a zone of decayed tissue adjacent to the healthy from which Pythium can not be isolated.

A clear picture of the relationship existing between the host and the parasite may be obtained from the study of material embedded in paraffin, sectioned, and stained with Delafield's haematoxylin and safranin. The fungus threads stain dark so that the outline of the hyphae and the granular contents of the mycelium are clearly pictured. The cell walls of the host also stain dark, but they are so much thinner than the mycelium that there is no possibility of confusing them. The cell contents stain a bright red. A careful examination of such preparations reveals the tissue composed of disconnected, collapsed cells, probably due in part at least to the loss of turgidity as a result of their death. The fungus invades the intercellular spaces and penetrates the cell wall, accumulating in the cells in considerable mass.

It is not known whether the cell wall is penetrated by mechanical pressure, by means of a dissolving enzyme, or by a combination of both. Hawkins and Harvey (*13*) seemed to be of the opinion that *Pythium debaryanum*, the cause of potato (*Solanum tuberosum* Linn.) leak, penetrated the cell wall by mechanical pressure and that no enzyme was associated in the process. In mottle necrosis of sweet potatoes the hyphae are greatly constricted at the point where they pass through the cell wall but after penetration enlarge to their original size. This fact further bears out the supposition that mechanical pressure is the method of cell-wall penetration, but does not necessarily preclude the possibility of enzymatic action. On this point Hawkins and Harvey (*13*) say: "If the fungus secretes an enzym which acts on the cell wall, it would seem probable that this enzym action would continue after the tip of the hypha passed through and the opening would be larger than the hypha. . . . With *Pythium debaryanum* on potato, however, the opening in the cell wall is never larger than the mean diameter of the hypha in the lumen of the cells and is usually considerably smaller."

There is nothing peculiar or distinctive about the mycelium within the cell. The hyphae usually enter the cell at right angles to the wall and continue in the same direction after penetration until they reach the opposite wall. Some of the mycelial threads, however, follow the inner wall and remain in close contact with it. In no case have hyphae been observed in contact with starch grains.

PHYSIOLOGICAL STUDIES

EFFECT OF TEMPERATURE ON MYCELIAL GROWTH

Because of certain irregularities in the growth in pure culture of *Pythium aphanidermatum*, *P. ultimum*, and *P. scleroteichum*, the three species with which most of the investigations were made, it was suspected that they possessed different temperature relations. This was particularly true of *P. scleroteichum*, which made a rather feeble growth at temperatures where *P. aphanidermatum* and *P. ultimum* thrived. A comparison of the growth of the three species on corn-meal agar in Petri dishes was therefore undertaken.

The methods employed are briefly as follows: Incubators with a range of temperatures varying from 2° to 41.8° C. and constant within 0.5° were employed. Ten cubic centimeters of corn-meal agar was poured into 9 cm. plates and distributed uniformly by

tilting from side to side while the agar was still hot. Plates with a thin covering of agar at the center were discarded. As soon as the medium had hardened a section 7 mm. in diameter was cut from a 2-day-old plate culture and placed at the center of the new plate. Five or more such plates were prepared for each organism for each temperature. The Petri dishes were inclosed within a covered moist chamber in order to prevent contamination with foreign organisms, of which there was very little within the duration of the experiments. In all cases where growth was interfered with by contamination the plates were discarded.

Pythium grows so rapidly that a Petri dish is often covered in less than 48 hours at the optimum temperatures. In view of this fact only two measurements were made and the optimum for growth
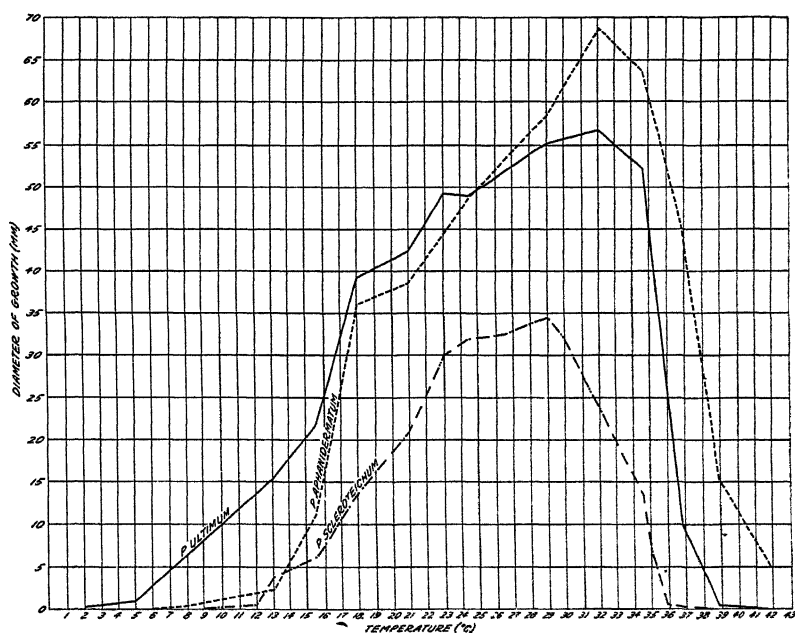
FIG. 3.—Influence of temperature at end of 24 hours on mycelial development of three parasitic species of Pythium grown in pure culture on corn-meal agar. (Measurements less than 1 mm. are estimates)

was assumed to be the temperature at which the greatest diameter of growth took place in 24 hours. The growth at the different temperatures is shown in Figure 3, the temperatures being plotted on the ordinate and the average diameter of the colonies in milli-meters on the abscissa.

A longer exposure to the different temperatures was necessary to establish the maximum and minimum for growth. The maximum and minimum were considered to be those extreme temperatures at which only a slight amount of growth took place. The question of time is, of course, an important factor in establishing the extremes, and particularly so at the lower temperatures. However, it was found that a temperature 1° to 2° C. above the maximum would kill the organism within a few days. At the lower temperatures

the plates were exposed for about six weeks, and, as would be expected, none of the organisms were killed. The optimum temperature for growth of *Pythium aphanidermatum* and *P. ultimum* was found to be 32° and of *P. scleroteichum* 29° (fig. 3). The highest temperature employed was 41.8°, where all the organisms were killed in 24 hours. *P. aphanidermatum* made a growth of 15.5 mm. at a temperature of 38.8°, while *P. scleroteichum* was killed and *P. ultimum* made scarcely any growth. The maximum for growth of *P. aphanidermatum* is probably slightly above 41.8°. While neither *P. ultimum* nor *P. scleroteichum* was killed at 36.8°, the growth was very slight, the former growing only 10 mm. and the latter just starting in 24 hours. These results indicate that a temperature of 36.8° was practically the limit for *P. scleroteichum;* that for *P. ultimum* was slightly higher. It is interesting to note in connection with the maximum temperature that a visible growth on the plate can not be interpreted strictly to mean that the organism will endure such temperatures. The death of the fungus at a killing temperature is not instantaneous. Pythiums grow very rapidly, and some growth would result before death would occur. The only safe criterion to follow is to determine if the organism will make additional growth when removed to a more favorable temperature. This method was followed, and the conclusions are drawn from the results so obtained.

The minimum temperature for growth was obtained by exposing cultures of the different species for 39 days at temperatures of 2.5° and 8° C. As a result it was found that in no case were any of the organisms killed, as was shown by their rapid growth when removed to a warmer temperature. *Pythium aphanidermatum*, with a maximum higher than either of the other species, has a minimum of 6°; *P. ultimum*, 2°; *P. scleroteichum*, about 8°. *P. scleroteichum*, with an optimum (29°) 3° lower than the other two species, has the narrowest range of growth, that of *P. ultimum* and *P. aphanidermatum* being about the same. The greatest diameter of growth over a 24-hour period was produced by *P. aphanidermatum* and the least by *P. scleroteichum* (fig. 3).

INFLUENCE OF TEMPERATURE ON AMOUNT OF DECAY

Investigations have shown that the optimum for the growth of *Pythium ultimum* is approximately 32° C., the minimum about 2°, and the maximum about 36.8°. A priori, it might be assumed that the optimum for the growth of the organism in pure culture would be approximately the same as that which would give the maximum amount of decay in a given time. This, however, appears not to be the case, at least so far as *P. ultimum* is concerned. As this organism is most frequently isolated from mottle-necrosis sweet potatoes, it was used in these investigations.

A preliminary experiment was made with the Triumph variety in order to acquire some information with respect to the probable location of the critical temperatures. Ninety-six sweet potatoes of this variety were inoculated in the usual manner, apportioned into 6 lots, and held for 5 days in incubators, the temperatures of which ranged from 5° to 35° C. At the end of this time the potatoes were removed from the incubators, cut open longitudinally, and measurements made of the spread of decay. The results are given in Figure 4.

and show that the spread of the decay increases with the rise in temperature up to 12°. Above this temperature the amount of decay decreases up to about 35°, where there is merely a trace of rotting. In another experiment, 20 inoculated sweet potatoes of the Triumph variety were held at 38° for 5 days, and no signs of decay appeared (fig. 4). From these data it may be concluded that *Pythium ultimum* may infect the Triumph variety over a wide range of temperatures (5–35°), with a minimum and a maximum slightly below and above those figures, respectively, and an optimum of about 12°.

As the available supply of the Triumph variety was insufficient, and as its normal shape—fairly long and cylindrical—did not lend itself readily to quantitative study, it was deemed best to change to the Yellow Jersey, a variety which is normally fusiform to globular or ovoid. The experiments with the Triumph variety, however,
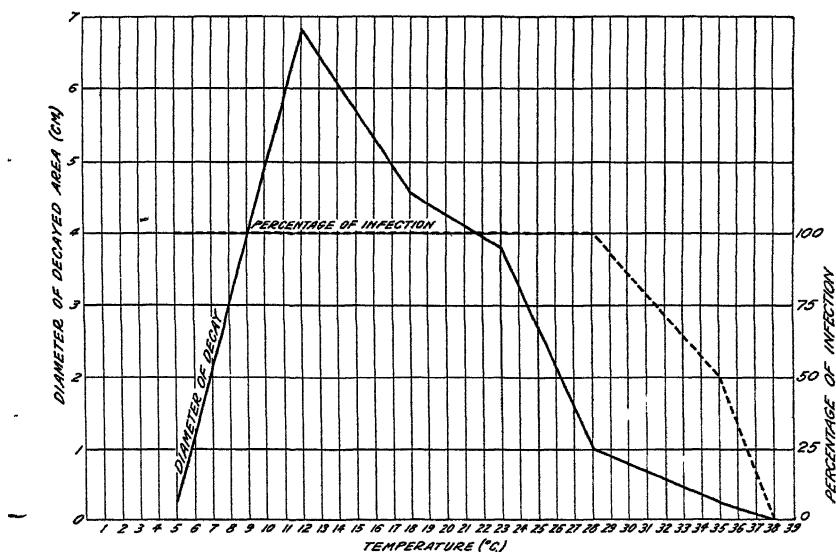
Fig. 4.—Influence of different temperatures on diameter of decayed area and percentage of infection of Triumph sweet potato inoculated with *Pythium ultimum*, after five days

served as a guide in the setting up and carrying out of later experiments. All the conclusions drawn and the data used in plotting Figure 5 are taken from two parallel sets of corroborating experiments made with the Yellow Jersey variety.

The experiments on the amount of decay were conducted as follows: Fairly large chunky sweet potatoes of as near uniform size as possible were selected and then cleaned by washing in water, after which they were inoculated by the same method as that employed in the pathogenicity tests, except that the well was made a little deeper in order that the inoculum might be placed as near the center of the sweet potato as possible. Ten sweet potatoes so treated were placed in wire baskets and exposed to temperatures ranging from 2° to 36° C. The duration of the experiment was three days, at the end of which time the potatoes were removed and weighed. They were then cut open, the decayed tissue was removed, and the healthy portion

weighed.   The amount of decay from all the sweet potatoes at a
given temperature was used as a basis for computation.   The tem-
perature at which the greatest total amount of decay occurred was
regarded as the optimum temperature.   To determine the maximum
and minimum temperatures, separate experiments were set up and
the time of exposure was increased; at the lower temperature the
time was increased to several weeks.   At the higher temperature
decay resulted within a few days or not at all, or the sweet potato
was rotted by some other organism.
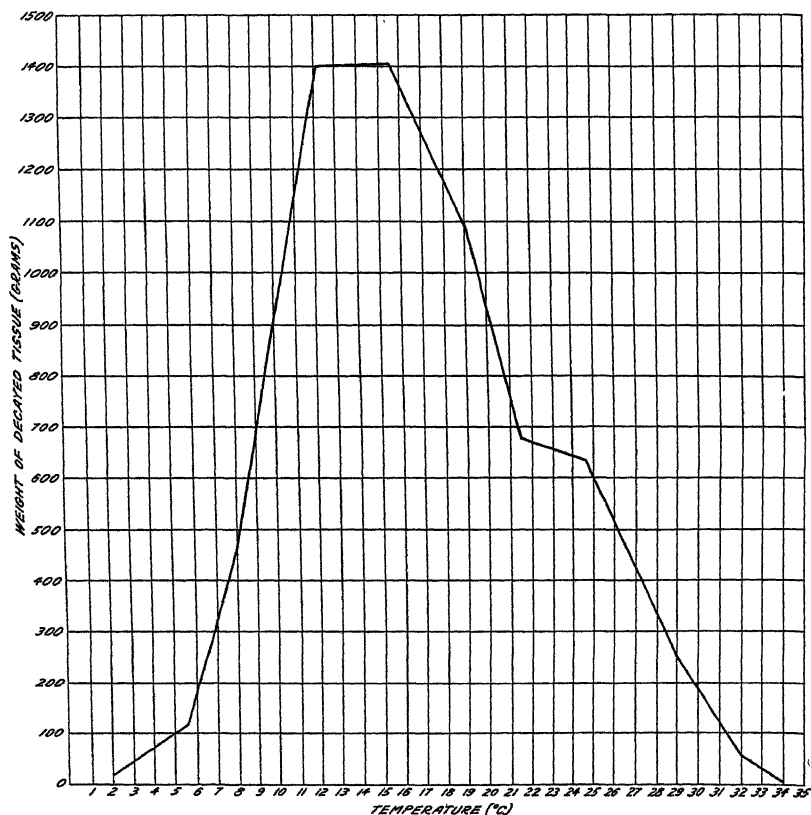
FIG. 5.—Decay (in grams), at different temperatures, of Yellow Jersey sweet potatoes inoculated with *Pythium ultimum*, after three days

The temperatures in the incubators did not vary more than 1
degree during the entire course of the experiment except in one incu-
bator which dropped several degrees for a few hours as the result of a
breakdown in the mechanical equipment.   This mishap, however,
did not seem to alter the results to any perceptible degree.   The
temperatures were read twice daily.

The temperatures employed and the amount of decay at each are
shown in Figure 5.   The results, especially at the optimum, are in
sharp contrast to what might be expected if the growth in pure agar
cultures is used as a guide.   A little decay took place at 2° and 34°
C., but as these temperatures are never maintained in a storage house,
they are of little practical consequence.   The amount of decay rose

, sharply with the increase in temperature to 12° and slightly more to 15.5°. The optimum, therefore, probably lies somewhere between 12° and 15.5°. From 15.5° the amount of decay decreases with the rise in temperature and amounts to practically nothing at 34°. This organism, with an optimum for growth of about 32° in pure cultures on cornmeal agar, produces the optimum amount of decay at a temperature much lower (12° to 15.5°). These results suggest that it is unsafe to draw any general and sweeping conclusions with respect to the optimum temperatures for decay from growth in artificial culture media. Each will need to be determined separately.

From the results of these investigations, it is apparent that the minimum temperature for infection is approximately 2° C.; the optimum, 12° to 15.5°; and the maximum, 34° to 35°. The minimum, optimum, and maximum are practically the same for infection of the Triumph and Yellow Jersey varieties of sweet potatoes.

In the infection tests conducted in the laboratory at room temperature (22–25° C.), it was noted that the same symptoms were not always produced when parallel experiments were carried out with the same organism. This was true with both *Pythium ultimum* and *P. scleroteichum*, and at times both produced the marble and the cheesy type of decay, a phenomenon which upset the a priori conception of the causal relationship of these organisms to mottle necrosis. In further defense of the nonspecificity of these two organisms for different types of decay, the studies on the effect of temperature on the amount of decay produced show that there is a correlation between the temperature and the type of decay produced. At temperatures from 2° to about 18° C. a cheesy type of rot was produced and the color was almost identical with the healthy tissue. The cavity produced was more or less uniform in diameter, with relatively smooth walls. Above 18°, however, the decayed tissue was more or less crumbly and mealy, a condition which became more pronounced with the rise in temperature. At the higher temperatures cavities of irregular shape and sizes were produced and the walls were more or less ragged and shredded.

These experimental data have a direct bearing on what may be expected, and what actually does happen, in the field. Experience has shown that the type of decay is more or less correlated with the temperature of the soil. If the weather is warm, as is likely in late summer, the marble type will result. If, on the other hand, the weather is cool following infection, the cheesy type will predominate. It was shown by several series of inoculation experiments with the same organism that the higher the temperature the greater the tendency to marbling. There was, as would be expected, an intermediate temperature at which either one or both might occur. At the optimum temperature for decay the cheesy type predominates, indicating that the marbling, as found in the field, occurs as a result of abnormal environmental conditions. Inasmuch as the optimum temperature for decay is low, the absence of the disease in some seasons might be explained on the basis of the high temperatures sometimes prevailing in the summer and fall. Further evidence of this is the fact that most of the initial infections which usually occur in late summer or early autumn take place at the lower ends of the edible roots, which are embedded in the cooler rather than in the upper warmer strata of soil.

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STORAGE

While any sweet potatoes noticeably infected with mottle necrosis would be discarded in digging time, some are likely to be overlooked and therefore would find their way into storage. Not only has a careful study been made of this kind of material, but mottle-necrosis material has been collected and exposed to different temperatures and humidities. For example, diseased potatoes have been stored under normal conditions, where the temperatures and humidity would fluctuate to some extent. At digging time (October 12 to 15) infected sweet potatoes also have been exposed to a temperature of from 22° to 25° C., with a relative humidity of about 90, and in an incubator with a temperature varying from 9° to 10°. At frequent intervals thereafter some of the sweet potatoes were removed and cultures made from diseased tissue. In the early part of the storage period Pythium could be isolated with ease, but, as time went on, this became increasingly difficult. Some of these sweet potatoes were kept in storage until the following March, but Pythium was not isolated later than November 13. It would seem from these results that the organism does not live long in dormant roots under storage conditions. Numerous microscopic examinations of necrotic tissue revealed the presence of a Pythium in and about the cells, but it lacked the appearance of a living organism. As it became more difficult to isolate Pythium, other fungi—such as Fusarium and Trichoderma—were obtained with greater ease and frequency. These latter organisms had apparently invaded the tissue decayed by Pythium and, it is not improbable, may have destroyed it. Certain results seem to indicate that Pythium is relatively short-lived when grown on artificial media; also that it is very sensitive to toxins such as might be released by other fungi growing in competition with it. At lower temperatures (9–10°) Pythium was isolated up to November 6. After this date *Mucor racemosus* Fes. only was obtained. This organism frequently causes considerable damage to sweet potatoes at low temperatures when the relative humidity is fairly high.

Whether or not any additional decay in storage is caused by Pythium has not been definitely determined. It is believed that there is little or none. Although Pythium in pure culture will rot sweet potatoes with ease, it seems to succumb readily to the competition of other fungi. While mottle-necrosis sweet potatoes have been examined during the storage period, no data have been collected with respect to the increased amount of decay by Pythium under natural storage conditions. In fact, such data would be difficult to obtain because of the presence of other fungi and the difficulty of measuring the amount of decay at different times without destroying the specimen. There is reason to believe, however, that other fungi, Fusarium, for example, enter mottle-necrosis tissue and continue to decay the sweet potato. Potatoes affected with mottle necrosis after a few weeks in storage often reveal a condition scarcely typical of the disease. The chambers and armlike ramifications through the potato characteristic of mottle necrosis are often no longer present. In other words, the sweet potato frequently is uniformly decayed from one end or one side in a manner resembling end rot caused by a Fusarium, or, if the temperature is rather low, by *Mucor racemosus*, which causes symptoms closely resembling those produced by Pythium.

In general, the writers are strongly of the opinion that Pythium is not a storage-rot-producing organism, but that if sweet potatoes infected in the field are stored the decay probably is continued by other fungi.

CONTROL

From the present knowledge of mottle necrosis, some recommendations which should help to control the disease can safely be made. The prevalence and destructiveness of the disease depends more or less on certain environmental conditions such as soil moisture and temperature. The disease is worse in certain types of soils, particularly in the medium light ones. It has been shown that the rootlet rot, caused by the same organism as mottle necrosis, occurs in the hotbed and that it is increased by using the same soil year after year. In view of these facts, it is advisable to use new soil or sand each year in the hotbed. Pythium succeeds best in soil or sand containing a considerable amount of moisture. Care should be exercised, therefore, not to apply any more water to the plants in the hotbed than is actually necessary. During the several years that these investigations have been under way the same ground in the field has been used for two or three years in succession. The results of this practice seem to have actually increased Pythium in the soil. Of course there is no actual proof of this, as the prevalence of the disease in the field is dependent—partially at least—on weather conditions, which were probably not identical for the different years. Nevertheless, where this disease has been destructive, it is advisable to plant the ground to other crops.

Ever since investigations with this disease were undertaken attention has been given to the comparative susceptibility and resistance of the sweet-potato varieties to mottle necrosis. Results recorded above have shown that all the varieties can be infected artifically. On the other hand, these varieties have been planted on infected soil, with the result that a considerable degree of resistance on the part of some of them was shown under field conditions. The Triumph, Yellow Jersey, and Big Stem Jersey have proved to be the most susceptible, and a little mottle necrosis has been found on the Georgia and Gold Skin in the field, but for the most part the remaining varieties listed in Table 2 may be regarded as immune, at least for all practical purposes. There is always, therefore, the possibility of substituting some of the immune varieties when the susceptible ones can not be grown.

RHIZOCTONIA

While the purpose of this paper is to discuss a disease of sweet potatoes caused by Pythium, it seems desirable, in order to avoid confusion, to discuss a similar condition in the field caused by *Rhizoctonia solani* Kühn. Attention has already been called to the prevalence of Pythium on the small roots and rootlets in the field and in the hotbed. During the summer of 1925, and to a lesser extent in 1924 and 1926, the roots were badly diseased in the sandier portion of the field. So far as macroscopic appearance went, the disease somewhat resembled Pythium rootlet rot, and it was so diagnosed. However, as a result of a large number of isolations it was found that *R. solani* was quite as prevalent as Pythium. Attention is called to

the presence of Rhizoctonia in order to avoid confusion in the diag-nosis of the disease in the field. When it was learned that both organisms were present, it was possible in some cases to separate the two. *R. solani* causes an almost black soft decay of the roots from the tip to the main stem. The cortex of the root is loosened and often can be separated easily from the xylem. Pythium, on the other hand, usually begins at the root tip. It attacks, as a rule, smaller roots than does Rhizoctonia. The death of many roots from attacks of Rhizoctonia often results in the development of a large cluster of small roots, roughly resembling a witches' broom (fig. 6).

Rhizoctonia solani, regardless of how prevalent it may be in the field, has not been found associated with mottle necrosis in any way.

SUMMARY

A field disease of sweet potatoes (*Ipomoea batatas* Poir.), known as mottle necrosis, has been described. The disease occurs very commonly on the Triumph, Yellow Jersey, and Big Stem Jersey, and, to a lesser extent, on some of the other common commercial varieties. When conditions are favorable for the disease as much as 40 to 50 per cent of the crop may be destroyed.

Several species of Pythium will produce mottle necrosis. Inoculation experiments have shown that it can be produced readily by *Pythium ultimum* Trow, *P. scleroteichum* Drechsler, and *P. aphanidermatum* (Edson) Fitzpatrick, and to a lesser extent by *P. splendens* Braun and *P. 5825*. *Pythium ultimum* and *P. scleroteichum* were isolated from rootlets in the hotbed. Therefore, the same organisms which cause rootlet rot cause mottle necrosis.

In the field three varieties of sweet potatoes (Triumph, Yellow Jersey, Big Stem Jersey) are very susceptible. All varieties tried were infected artificially.

The type of decay depends largely on the temperature. If the temperature is 20° C. or above, it is likely to be marbled; if lower, the cheesy type of decay will predominate.

The optimum for the growth of *Pythium ultimum* and *P. aphanidermatum* in pure culture on corn-meal agar is 32° C.; for *P. scleroteichum*, 29°. The minimum temperatures are approximately as follows: *P. ultimum*, slightly below 2°; *P. aphanidermatum*, about 6°; *P. scleroteichum*, about 8°. The maximum temperature for *P. ultimum* is approximately 41.8°, for *P. scleroteichum* about 38.5°, and *P. aphanidermatum* about 41.8°.

The results of these investigations show that, although the optimum temperature for growth of *Pythium ultimum* in pure culture is about 32° C., the greatest amount of decay of sweet potatoes is produced in a given time (five days) at temperatures between 12° and 15°. The optimum temperature for growth in pure culture and the optimum temperature for decay are quite dissimilar.

Mottle necrosis has not been found to be a storage trouble.

Rhizoctonia solani Kühn has been found to cause decay of some of the small roots of sweet potatoes in the field. The symptoms produced resemble somewhat rootlet rot caused by Pythium, but differ from the latter in that the roots are more completely destroyed, and also in that clusters of small roots subsequently develop which re-

⌐semble a witches' broom. Rhizoctonia seems to be more prevalent on light sandy soils. It is in no way associated with mottle necrosis. Crop rotation should be practiced where control measures are required. New soil should be used each year in the hotbed. While

FIG. 6.—Excessive root development, resembling a witches' broom, on sweet potato, probably caused by *Rhizoctonia solani*. (About three-fifths natural size.)

all the commercial varieties have been infected artificially, only the Triumph, Yellow Jersey, and Big Stem Jersey have been found seriously diseased in the field. A number of good commercial varieties appear to be more or less immune.

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THE COMPARATIVE SUSCEPTIBILITY OF SWEET-POTATO VARIETIES TO STEM ROT [1]

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INTRODUCTION

Investigations [2] have shown that sweet potatoes (*Ipomoea batatas* Poir.) are affected with a disease known as stem rot or wilt (caused by *Fusarium batatatis* Wr. and *F. hyperoxysporum* Wr.), whose distribution throughout the United States is more or less correlated with the distribution of the susceptible varieties. In some regions of the South stem rot is unknown or causes little or no loss, owing to the fact that the varieties of sweet potatoes there grown are immune or highly resistant and suffer no apparent injury from it. On the other hand, in the northern tier of the sweet-potato-growing States localities may be found where stem rot is so severe on the small number of varieties grown—and practically all of them are very susceptible to the disease—that it threatens to destroy the industry or to force it out of its present centers.

Soil, climate, and commercial competition with other crops have confined the sweet potato in the northern range to relatively small areas in several States (New Jersey, Delaware, Maryland, Virginia, Ohio, Indiana, Illinois, Iowa, Kansas, and California) where a susceptible variety is generally grown, sometimes for 3 to 12 or 15 years on the same soil without rotation with other crops. Not only is this a bad agricultural practice but it causes the soil to become thoroughly infested with the causal organisms. All of the plants being potential hosts, many of them become actually infected.

In the South the situation is somewhat different. A large number of varieties are grown there, many of which are apparently resistant to stem rot or are not injured by it. If any particular variety is found to be damaged by the disease, a resistant one, probably equally acceptable to the consumer, can be substituted for it.

The disease situation has become so serious in some of the Northern States that a series of experiments were undertaken which had for their object to determine, first, the comparative susceptibility of a number of commercial varieties and, second, the adaptability of southern-grown varieties to northern conditions with the view of selecting varieties showing a suitable degree of resistance to stem rot. Not all the known varieties of sweet potatoes were tried in these experiments, the collection consisting of those of established economic

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importance and others of commercial promise. The following vari-eties were used: Big Stem Jersey, Creola, Dahomey, Dooley, General Grant Vineless, Georgia, Gold Skin, Haiti, Key West, Nancy Hall, Pierson, Porto Rico, Pumpkin, Red Brazil, Red Jersey, Southern Queen, Triumph, White Yam, Yellow Jersey, Yellow Strasburg, Yellow Yam.

In 1914 Harter and Field [3] studied the susceptibility of several varieties of sweet potatoes to stem rot by making artificial inoculations in the field. A few varieties were also planted on infested soil in New Jersey. The results of these several experiments showed that a number of the varieties could be infected artificially; also, that natural infection of a few varieties occurred when planted on infested soil. Inasmuch as plants often may be inoculated artificially when, under normal field conditions, they might remain healthy, it was planned to conduct a series of field tests, using, where practicable, only naturally infested soil.

METHODS OF EXPERIMENTS

A 1-acre plot was selected at Houston, Del., and the varieties were grown on this field for four consecutive years, beginning with 1922. Another plot of a similar area at Seaford, Del., where stem rot was very prevalent, was planted to the same varieties for two consecutive years, 1925 and 1926. Stem rot, though present, was not especially abundant in the Houston soil, and in order to increase the chances for infection and infestation of the soil the roots of the plants used in 1922 and 1923 were dipped in a spore suspension of the stem rot organisms just previous to planting. About 500 plants of each variety were set out at both Houston and Seaford. Counts of diseased and healthy plants were made about every 10 days to 2 weeks from the time of setting until the vines covered the field so that the individual hills could no longer be recognized. At each count the diseased plants were pulled up and removed from the field. In the fall when the crop was dug each individual plant was carefully examined for stem rot.

The original plan of this variety test called for a five-year planting on the 1-acre plot selected at Houston, Del. Because of the depredations caused by chickens and other barnyard animals, it was thought advisable to check the results by duplicating the experiments for two years at Seaford, Del. The latter plot was located in the center of a field where a large percentage of the plants were infected the previous year. The soil, ideally suited to the growing of sweet potatoes, was well prepared and the crops received the best of care throughout the growing season. Notwithstanding the fact that conditions on the Houston plot were not of the best, the results, when compared with those obtained at Seaford, are strikingly similar.

The seed potatoes of the different varieties, with two or three exceptions, were obtained from the Office of Horticulture, Bureau of Plant Industry, United States Department of Agriculture. The plants were grown at the Arlington Experiment Farm near Rosslyn, Va., and shipped by express to Houston and Seaford when required.

[3] Harter, L. L., and Field (Tillotson), E. C. Experiments on the susceptibility of sweet potato varieties to stem rot. Phytopathology 5: 163–168. 1915.

EXPERIMENTAL DATA

Figure 1 shows in graphic form the reaction of different varieties of sweet potatoes to stem-rot infection at both Houston and Seaford, Del. The curves shown in Figure 1 were plotted from the combined
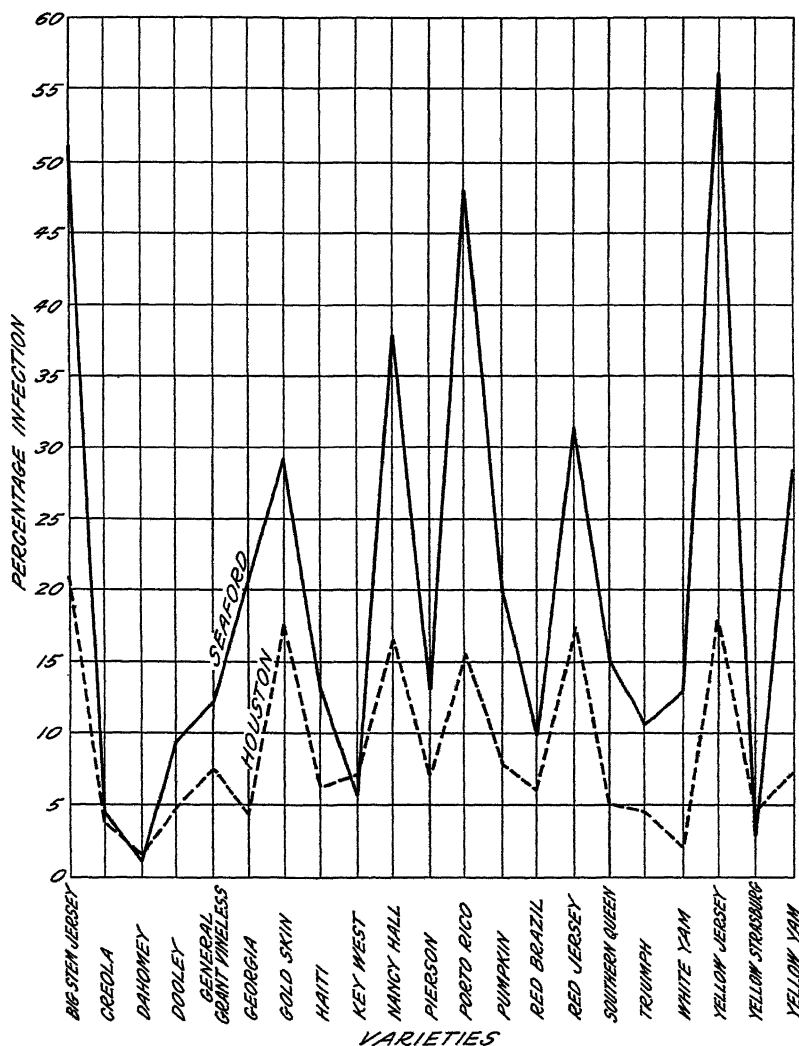
FIG. 1.—Percentage infection of different varieties of sweet potatoes by the stem-rot organisms for the period of experiment—four years at Houston and two years at Seaford, Del.

results of a four-year and a two-year test at Houston and Seaford, respectively. The total number of plants of each variety grown at each station and of those diseased (including both those removed during the season and at the final harvest count) was used as a basis for calculating the percentage of infections. As would be expected, there was some variation from year to year among the varieties, and

during seasons especially favorable to infection the percentage of diseased plants was noticeably higher.

A careful examination of these curves shows that there is, for the most part, a marked degree of similarity of infection of the same varieties grown at the two stations. One notable exception is the Georgia variety which shows 4.5 per cent infection at Houston and 27.5 per cent at Seaford. The results at Houston are surprising, in view of the fact that this variety has been found quite susceptible when observed in a number of different locations.

Special attention should be called to the results of two other varieties, White Yam and Haiti. Both of these varieties usually exhibit a high degree of resistance to stem rot, the percentages at Houston being 2.1 and 6.3, respectively. At Seaford, on the other hand, in a badly infested soil, the infection increased to 13.1 and 13, respectively, on the same varieties.

An examination of the curves shows that the percentage of infection of the same varieties is, in general, higher at Seaford than at Houston. Six varieties stand out as especially susceptible to stem rot, namely, Yellow Jersey, Big Stem Jersey, Porto Rico, Nancy Hall, Red Jersey, Gold Skin, and Georgia. Yellow Jersey and Big Stem Jersey, with percentages of infection of 56.3 and 51.1, respectively, at Seaford, are followed by Porto Rico and Nancy Hall. There is nothing surprising in these results, for the varieties are those that have long been recognized as the most susceptible.

It is evident, however, that none of the varieties tried in these experiments are entirely immune. Poole[4] found Red Brazil and Triumph to be practically immune to stem rot under New Jersey conditions, there being either no plants killed or infections were less than 1 per cent. The Triumph was found to be highly resistant. It showed only 2 per cent diseased plants during the season.

Judging from the results of the writers' investigations, none of the 21 varieties studied in these experiments are entirely immune to stem rot. Obviously cultures were not made from the thousands of plants examined. Whether or not a plant was infected was based entirely on the characteristic macroscopic appearances of the plants. During the several counts in the summer following planting, infection was determined mostly from the general appearances of the foliage and occasionally from the discoloration of the fibrovascular bundles at the soil line. When the diseased plants were counted and removed at this time only those unquestionably infected were classed as diseased, the doubtful ones being left for observation at the next count. All the varieties showed a certain percentage of infection at these early counts and some of the plants of more or less resistant varieties were killed. The percentage of killed plants, however, is considerably smaller than Figure 1, which includes those barely infected as well as those actually killed, indicates. The fact that a few plants of all varieties are actually killed is proof of the statement already made that none of the varieties are actually immune.

Just when a plant is or should be classed as infected is sometimes difficult to tell, and it is not unlikely that different observers well

[4] POOLE, R. F. THE STEM ROT OF SWEET POTATOES. LOSSES, SOURCES OF INFECTION AND CONTOL. N. J. Agr. Expt. Sta. Bul. 401, 32 p., illus. 1924.
——— CULTURAL METHODS FOR REDUCING SWEET POTATO LOSSES CAUSED BY STEM ROT. N. J. Agr. Expt. Sta. Bul. 433, 16 p., illus. 1926.

acquainted with the disease would not always agree. On the one hand there are the unmistakable symptoms such as death of the plant and the blackening of the fibrovascular bundles about which there can be no question, but on the other hand there are those doubtful cases which are on the border line whose classification rests wholly upon the judgment of the observer. At the time of digging there was a large number of such border-line cases especially among those varieties exhibiting on the whole a considerable degree of resistance (Dahomey, Triumph, Haiti, Red Brazil, White Yam, and others). When the plants were examined in the fall, the roots of each plant were cut open with a knife. In some cases it was found that the causal fungus had gained entrance at the wound made by pulling the plant from the mother potato and had extended for 1 or 2 inches up into the stem. It was also found that the stem-rot fungus would sometimes enter through a soil-rot (pox) lesion and invade the fibrovascular system for a short distance in either direction or that it would enter through other channels, such as wounds made in cultivation or by other means. It is interesting to note in this connection that there were no external symptoms of stem rot. The foliage was healthy, and the plants grew normally and produced a normal crop. A large percentage of the more or less resistant plants responded in this way, and in those cases where a plant was actually killed the infection took place in the spring soon after the plants were set out and before they had become established. There is, then, a number of varieties of this type which, while not entirely immune, are resistant enough to yield a normal crop on badly infested soil. The varieties that could be safely planted on infested soil are as follows: Creola, Dahomey, Haiti, Key West, Pierson, Pumpkin, Red Brazil, Southern Queen, Triumph, White Yam, and Yellow Strasburg.

SUMMARY

The relative susceptibility of 21 varieties of sweet potatoes to stem rot (caused by *Fusarium batatatis* Wr. and *F. hyperoxysporum* Wr.) was tested four years at Houston, Del., and two years at Seaford, Del., on naturally infested soil. At Houston the infestation of the soil and the chance for infection were increased by dipping the roots of the plants in a spore suspension of the stem-rot organisms just previous to planting.

The two curves (fig. 1), which are more or less strikingly parallel, show that those varieties very susceptible at Seaford were also very susceptible at Houston.

Judging from the results of these experiments, there are no varieties entirely immune to stem rot, but such varieties as Creola, Dahomey, Haiti, Key West, Pierson, Pumpkin, Red Brazil, Southern Queen, Triumph, White Yam, and Yellow Strasburg are so slightly injured by the invasion of the parasites that a normal crop can be produced by them even when they are grown on badly infested soil.

The following varieties are very susceptible to the disease: Yellow Jersey, Red Jersey, Porto Rico, Nancy Hall, Gold Skin, Georgia, and Big Stem Jersey. A large percentage of the infected plants of these varieties are killed during the growing season.

INHERITANCE OF RATE OF SHEDDING IN A COTTON HYBRID [1]

By Thomas H. Kearney, *Senior Physiologist in Charge*, and Robert H. Peebles, *Senior Scientific Aid, Office of Alkali and Drought Resistant Crops, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The tendency of the cotton plant to shed many of its flower buds and young bolls is so important in its relation to cotton production that no aspect of this phenomenon should be disregarded. Variations in the rate of shedding within a variety doubtless are determined chiefly by environmental factors, including such external biological factors as insects and diseases. But when different types of cotton are compared as to their shedding percentages, the probability that genetic factors also are involved must not be overlooked.

The writers have presented evidence that different species and varieties of cotton differ consistently in the rate of shedding. They have shown also that in a hybrid between Pima Egyptian and Acala upland cottons the second generation was more variable than the first and that individual F_2 plants grown under identical conditions differed significantly in the percentage of buds and of young bolls lost by abscission.[2] Third-generation progenies have been grown subsequently, and the data obtained from them are set forth in the present paper. The new evidence confirms the conclusion that abscission of the flower buds and young bolls in cotton is determined partly by genetic factors.

COMPARISON OF F_3 PROGENIES WITH THEIR F_2 PARENTS

The investigation was conducted at the United States Field Station, Sacaton, Ariz. Fifteen individuals of the second generation of the hybrid Pima × Acala were selected in 1925, of which five had shown a high rate of shedding, five an intermediate rate of shedding, and five a low rate of shedding. Third-generation progenies were grown in 1926 from self-pollinated seed of the F_2 plants, and a record was kept for each plant of the total number of flower buds and flowers produced and of the losses by abscission, both before and after anthesis. The method of taking the shedding records is described by the writers in a previous paper.[3] From the resulting data were computed the shedding percentages of the individual plants and the mean and standard deviation of the mean of each F_3 progeny.

The mean shedding percentages of each progeny were computed by taking, for all plants as one array, the number of flower buds shed, the number of young bolls shed, and the number of buds shed plus the number of bolls shed as percentages of the total number of flower buds produced; also the number of bolls shed as a percentage of the number of flowers (buds which reached the stage of anthesis). The standard deviations of these percentages were computed with regard to variation among the individual plants of the progeny by the formula

$$\sigma = \sqrt{\frac{\Sigma n p^2}{\Sigma n} - M p^2}$$

where p = the shedding percentage of each plant, n = the number of buds or of flowers produced on each plant, and Mp = the mean shedding percentage of the progeny. The standard deviations computed by this formula were then increased to take account of the small numbers of plants in these populations, and the probable errors were computed from the standard deviations as thus corrected.[4] The standard deviations as given in Tables 1 and 2 have been further increased to bring them to the values expected had the ratio been 0.5 or 50 per cent,[5] thus making the standard deviations of large and of small percentages comparable as expressions of the variability of the several populations. This second correction was not used in computing the probable errors as here given, although it was erroneously so used in the paper cited.

The shedding percentages of the F_2 parents and the statistical constants of the F_3 progenies are given in Table 1, in which all percentages are based upon the total number of flower buds produced by the F_2 plant or the F_3 progeny; and in Table 2, in which the boll-shedding percentage is based upon the total number of flowers produced by the F_2 plant or the F_3 progeny.[6] The progenies are arranged in both tables in the order of the total shedding percentages of the F_2 parents.

[4] In computing the probable error by the formula $E = .6745 \frac{\sigma}{\sqrt{n}}$, n was taken as the number of plants in the progeny, and not as the number of buds or of flowers.

[5] KEARNEY, T. H., and PEEBLES, R. H. Op. cit. p. 654, footnote.

[6] On seven of the F_2 plants the time of shedding, whether before or after anthesis, was not recorded for some of the "sheds," the number unclassified having constituted from 5 to 33 per cent of the total number of buds and bolls shed by these plants. The unclassified "sheds" were arbitrarily divided between buds and bolls in the proportion indicated by the known numbers of buds and bolls shed by the plant in question. The bud-shedding and boll-shedding percentages of these plants are correspondingly uncertain, but the peparture from the true percentage could in no case be large enough to affect the conclusions. The total shedding percentage is not affected by this uncertainty of classification

TABLE 1.—*Shedding percentages of 15 F₂ plants and their F₃ progenies of a hybrid between Pima Egyptian and Acala upland cottons, the number of buds and of bolls shed being taken as a percentage of the number of flower buds produced*

Designation of F₂ parent and F₃ progeny	Number of plants in F₃ progeny	Percentage shedding before anthesis (bud shedding)			Percentage shedding after anthesis (boll shedding)			Percentage total shedding (bud and boll shedding)		
		F₂ individual	F₃ progeny Mean	Standard deviation	F₂ individual	F₃ progeny Mean	Standard deviation	F₂ individual	F₃ progeny Mean	Standard deviation
High group.										
4-15-24	18	47 5	58.2±4.5	28.8	42.5	23.4±2.6	19.5	90.0	81.6±2.4	19.5
7-17-13	6	49.2	40.3±3.5	13.0	38.8	48.3±4.4	15.9	88.0	88.6±1.0	5 7
18-2-20	8	19.2	24.7±2.0	9.7	68.3	54.3±2.4	9.9	87.5	79.0±1.5	7.6
17-13-20	11	15 1	58.2±4.4	21.7	67.7	27.2±3.6	20.1	82.8	85.4±2.1	10.6
4-1-22	16	27.6	45.5±3.5	20.7	53.9	38.2±2.8	17.1	81.5	83.7±1.7	13.6
Average	------	31.7±5.1	45.4±4.5	------	54.2±4.4	38.3±4.2	------	86.0±1.2	83.7±1.2	------
Intermediate group:										
4-15-6	16	22.0	24.9±3.0	20.5	40.6	33.5±2.1	13 0	62.6	58.4±2.3	13.9
4-15-14	8	31.0	56.2±3.4	14.6	31.0	25.0±2.2	10.7	62.0	81.2±2.1	11.5
7-1-21	12	32.3	22 5±2.9	17.8	28.2	48.6±4.7	24.2	60.5	71.1±4.1	22.9
17-13-9	11	13.5	49.1±3.8	18 8	45.8	17.2±1.9	12.6	59.3	66.3±3.0	15.4
7-1-22	18	15.9	46.6±4.8	30.3	42.0	35.2±3.7	24.4	57.9	81.8±1.5	12.4
Average	------	22 9±2.7	39.9±4.9	------	37.5±2.4	31.9±3.8	------	60.5±0.6	71.8±3 2	------
Low group:										
17-16-12	15	10 8	28.0±3.3	21.3	29.4	27.9±2.3	14.6	40.2	55.9±3.1	17.7
7-1-1	14	19.8	56.7±2.8	15.8	19.8	10.1±1.3	11.6	39.6	66.8±2.2	12.7
17-13-3	18	6 0	29.9±2.8	19.2	29.8	23.1±1.9	14.3	35.8	53.0±2.2	13.8
17-13-12	13	8.7	29.2±4.8	28.4	26.3	45.3±3.7	20.0	35 0	74.5±1.7	10.5
18-7-10	11	3.9	34.0±4.0	20.8	18.0	29.1±3.6	19.5	21.9	63.1±4.4	22.5
Average	------	9.8±2.0	35.6±3.9	------	24.7±1.8	27.1±4.1	------	34.5±2.4	62.7±2.8	------

TABLE 2.—*Boll-shedding percentages of 15 F₂ plants and their F₃ progenies, of a hybrid between Pima Egyptian and Acala upland cottons, the number of bolls shed (abscissions after anthesis) being taken as a percentage of the number of flower buds which reached anthesis*

Designation of F₂ parent and F₃ progeny	Boll-shedding percentage F₂ individual	Boll-shedding percentage F₃ progeny mean	Standard deviation of F₃ mean
High group:			
4-15-24	81.2	56.0±3 6	17.8
7-17-13	76.6	80.9±3.8	17.7
18-2-20	84.5	72.2±1.9	8.9
17-13-20	79.8	65.0±3.8	19.4
4-1-22	74.6	70.1±2.7	17.3
Average	79.3±1.1	68.8±2.9	---------
Intermediate group:			
4-15-6	52.1	44.6±2.1	12.3
4-15-14	44.9	57.1±4.6	15.5
7-1-21	41.6	62.7±5.1	27.3
17-13-9	53.0	33.8±2.9	15.3
7-1-22	50.0	65.9±2.0	13 0
*Average	48.3±1.6	52.8±4.3	---------
Low group:			
17-16-12	32.9	38.7±2.9	17.1
7-1-1	24.7	23.3±2.3	15.3
17-13-3	31.7	33.0±2.6	17.1
17-13-12	28.8	64.0±2.0	11.2
18-7-10	18.7	44.1±5.0	24.9
Average	27.4±1.9	40.6±4.9	---------

The data given in Tables 1 and 2 show considerable variation within each group (high, intermediate, and low) both as regards the means of the F_3 progenies and the standard deviations of the means. When the groups are compared on the basis of averages,[7] however, a consistent relation is observed. The group of high-shedding F_2 plants gave the highest average in F_3 for bud-shedding percentage, boll-shedding percentage, and total shedding percentage, all based on total buds (Table 1) and for boll-shedding percentage based on total flowers (Table 2). Similarly, the group of low-shedding F_2 plants gave the lowest average in F_3 for all shedding percentages and the group of intermediate F_2's gave intermediate averages in F_3. The differences among the averages of the three groups of F_3 progenies are greatest in respect to total shedding based on total buds (Table 1) and boll shedding based on total flowers (Table 2). The difference in F_3 between the average of the high and the average of the low group was seven times its probable error for total shedding percentage (Table 1) and five times its probable error for boll-shedding percentage (Table 2).

There was little correspondence between the individual F_2 plants and their F_3 progenies in bud-shedding percentage and boll-shedding percentage based on total buds (Table 1), the coefficient of parent-offspring correlation having been, for the bud-shedding percentage 0.289 ± 0.160, and for the boll-shedding percentage as thus computed 0.272 ± 0.161. On the other hand, the coefficient of parent-offspring correlation (F_2 with F_3) for the total shedding percentage was high and significant, being 0.715 ± 0.085, or more than eight times its probable error. There was also a fairly high and significant parent-offspring correlation for boll-shedding percentage based on total flowers (Table 2) r being 0.621 ± 0.107.[8]

The data from F_3 therefore support the conclusion suggested by study of the F_2 population in 1925 that shedding is partly controlled by genetic factors which, following hybridization, segregate and recombine, although no definite Mendelian ratio is observable.

BUD SHEDDING IN RELATION TO BOLL SHEDDING

Further evidence of the incidence of genetic factors in shedding was sought by examining the relation between abscission before and after anthesis in the same populations. When the "sheds" occurring in both stages are taken as percentages of the total flower buds produced (Table 1), there was no significant correlation between the bud-shedding and boll-shedding percentages of the F_2 plants (r 0.166 ± 0.169), but the F_3 progeny means showed a rather high and very significant negative correlation ($r - 0.643 \pm 0.102$). This was to be expected in view of the fact that in F_3, in which the total shedding was in most cases heavier than in F_2, all of the progenies lost by abscission before and after anthesis (total shedding) more than 50 per cent of their flower buds. If most of the buds are shed before anthesis, obviously few will be left to shed after anthesis; and, conversely, heavy losses after the buds have reached anthesis implies

that comparatively few could have been lost before anthesis. The relation seems to be a purely mechanical one, although, as was pointed out in the earlier paper,[9] physiological factors also may be involved if, as is often assumed, the cotton plant normally produces more flower buds than it can develop into mature bolls. In this case, if the greater part of the "self-pruning" is accomplished before anthesis, less will be necessary after anthesis, and vice versa.

The influence of these mechanical or physiological relations is eliminated when the bud-shedding percentage (Table 1) is correlated with the boll-shedding percentage based upon the number of flowers instead of the number of flower buds (Table 2). When this was done, a positive correlation was found in both generations, the coefficients having been 0.613 ± 0.109 in F_2 and 0.346 ± 0.153 in F_3. The F_3 coefficient is only 2.3 times its probable error, but since it is positive like the certainly significant F_2 coefficient (r/E 5.6) there can be little doubt that there is a tendency, in both generations, for plants which shed many of their flower buds to shed also many of their young bolls, and vice versa. The occurrence of this positive correlation may be taken as additional evidence that shedding in these hybrid populations is determined partly by genetic factors.

SHEDDING RATE HEAVIER IN F_3 THAN IN F_2

By comparing the averages of the three groups for total-shedding percentages (Table 1) it is evident that the agreement between F_2 and F_3 is much closer in the high group than in the intermediate and low groups. In the high group the F_3 average is slightly lower than the F_2 average, while in the intermediate group F_3 gives a considerably higher and in the low group a much higher average percentage of total shedding than F_2. Similar relations are shown by the boll-shedding percentages based on total flowers (Table 2). In the high group the average for F_3 is somewhat lower than for F_2, while in the intermediate group F_3 gives a slightly higher average and in the low group a much higher average than F_2.

Shedding was so nearly complete in the high group of F_2 plants that their F_3 progenies could not be expected to show increases of the same magnitude as in the intermediate and low groups. But the absence of any increase at all in the averages for the high group is difficult to account for. An increased rate of shedding, if common to all three groups, would not have been surprising in view of the evidence from a similar interspecific cotton hybrid[10] that the average number of bolls retained per plant was much smaller for the F_3 progenies than for the corresponding F_2 parents.

Another explanation of the higher rate of shedding in F_3 than in F_2 would be afforded if it could be shown that seasonal conditions were more conducive to a high rate of shedding in 1926, when the F_3's were grown, than in 1925, when the F_2's were grown. If so, the difference should have been reflected in the rates of shedding of the parental varieties, Pima (Egyptian) and Acala (upland). No record of bud shedding of these varieties was made at Sacaton in 1926, but boll shedding was recorded on 100 plants of each variety by Harold F. Loomis of the Office of Cotton, Rubber, and Other Tropical Plants,

[9] KEARNEY, T. H., and PEEBLES, R. H. Op. cit. p. 658.
[10] KEARNEY, T. H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Bul. 1164, p. 42. 1923.

Bureau of Plant Industry. The resulting percentages, in comparison with those obtained by Mr. Loomis in 1925, are given in Table 3.[11] No significant difference between the two seasons is indicated by these data, and the only explanation remaining for the heavier shedding of the hybrid population in 1926 is that the tendency to sterility in this interspecific cotton hybrid is more pronounced in F_3 than in F_2.

TABLE 3.—*Boll-shedding percentages of the parental varieties, Pima (Egyptian) and Acala (upland)* [a]

Year	Pima variety			Acala variety		
	Number of—		Boll-shedding percentage	Number of—		Boll-shedding percentage
	Plants	Flowers		Plants	Flowers	
1925	20	1,004	11.2±3.62	20	945	66.7±1.83
1926	100	3,962	9.9±0.40	100	7,008	68.8±0.68
Difference			1.3±3.64			2.1±1.96

[a] The percentages were computed (as in Table 2) from the total number of flower buds reaching anthesis and the total number of "sheds" occurring after anthesis in each population. The probable errors of the percentages are computed from the squared departures from the mean of the population of the shedding percentages of the individual plants, each weighted by the number of flowers on the plant.

Comparison of the boll-shedding percentages of the hybrids (Table 2) with those of populations representing the parental varieties (Table 3) shows that for the high group the average in F_2 was higher than that of Acala grown in the same year (1925), while the average in F_3 was the same as that of the Acala population of 1926. For the low group the average boll-shedding percentage in F_2 was more than twice that of Pima grown in 1925, and the average in F_3 was four times that of Pima grown in 1926. None of the 155 plants of the F_2 population of 1925 had a boll-shedding percentage as low as the average of the Pima population of that year.

CONCLUSIONS

Study of the second generation of the interspecific cotton hybrid Pima Egyptian × Acala upland suggested that the shedding of flower buds and young bolls is determined partly by genetic factors. Conclusive evidence that such is the case was afforded by third generation progenies of F_2 plants which had shown, respectively, a high, intermediate, and low rate of shedding. The coefficient of parent-offspring correlation (F_2 with F_3) was 0.715±0.085 for the total-shedding percentage (buds and bolls shed as a percentage of total buds) and 0.621±0.107 for the boll-shedding percentage (bolls shed as a percentage of total flowers).

Further evidence that genetic factors are involved is given by the positive correlation between rate of bud shedding and rate of boll shedding in both F_2 and F_3 of the hybrid. This fact proves that the inherited tendency to a high or a low rate of shedding expresses itself in abscission both before and after anthesis.

There was a tendency to greater sterility in F_3 than in F_2, as was noted also in an earlier investigation of an upland-Egyptian cotton hybrid, but the data at hand do not suggest a satisfactory genetic explanation of this difference between the two generations.

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[11] The data in Table 3 accord with the results of previous years in showing a fairly consistent difference between Pima and Acala cottons in their rates of boll shedding at Sacaton. The ratio of the boll-shedding percentage of Pima to that of Acala was 1 to 4.5 in 1923, 1 to 4.2 in 1924, 1 to 6 in 1925, and 1 to 6.9 in 192.

THE BASAL METABOLISM OF MATURE CHICKENS AND THE NET-ENERGY VALUE OF CORN[1]

By H. H. MITCHELL and W. T. HAINES, *Illinois Agricultural Experiment Station*

INTRODUCTION

One of the great contributions of Armsby to the science of animal nutrition was the demonstration that the requirements of animals for nutrients, and the values of feeds in covering these requirements, are two separate and distinct problems, requiring different experimental methods for their solution. The requirement of an animal for energy, for example, must involve a study of the heat production of the animal and of the energy content of animal tissues and secretions produced during growth, fattening, egg production, gestation, and lactation. The energy requirements are, therefore, best expressed in terms referring to the animal rather than to the feed; hence the measurement of animal requirements in terms of feed or digestible nutrients is an incomplete and an inaccurate measurement, however valuable it may be in practical animal husbandry.

According to Armsby (1),[2] the measurement of the true value of feeds as sources of energy to animals involves a determination of the energy content of the feed and of all losses of energy incurred in its utilization by the animal. If the latter are subtracted from the former, the "net-energy" value of the feed is obtained. A more complete definition of the actual energy value of a feed to the animal has never been proposed, nor one of greater significance as a guide to experimental investigation. It would seem that the same conception might be applied to other nutrients.

WORK OF OTHER INVESTIGATORS

While considerable work has been done upon the net-energy value of feeds for steers, practically no work of this character has been done with reference to other farm animals. The present investigation was undertaken as an initial study of the utilization of feed energy by chickens.

Gerhartz in 1914 reported (2) an extensive investigation on two hens concerned with the energy required in egg production. As one phase of the investigation some consideration was given to the question of the "work of digestion" of food, i. e., the loss of food energy as heat during digestion and assimilation. Upon comparing the increase in heat production of hens during a day in which food was consumed over that during a day of fast, with the intake of nutrients, Gerhartz concluded that Zuntz's factors for the estimation of the "work of digestion" could be applied to chickens.[3] However, the conclusion appears to be very poorly supported by the experimental data compared, since on the days of fast the heating effect of the

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[1] Received for publication Feb. 3, 1927; issued July, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 943.
[3] Zuntz's factors (*2, p. 189*) obtained upon mammals are: 0.8 calorie per gram of protein consumed, 0.24 calorie per gram of fat, and 0.4 calorie per gram of carbohydrate.

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(927)

meal consumed on the preceding evening can not be regarded as having been entirely dissipated in 10 to 20 hours, while on the days of feeding the full heating effect of the food can not be assumed to have been obtained in an experimental period of less than 12 hours' duration. In one of the feeding periods the hen consumed an egg laid only three hours before the termination of the respiration period; nevertheless, the full heating effect of the contained nutriment is assumed to have been obtained. In view of these facts, the close agreement that Gerhartz obtained in two comparisons between the observed increases in heat production and those computed by means of the Zuntz factors applied to the nutrients consumed must be considered purely fortuitous.

Hári (*4*) and Hári and Kriwuscha (*5*) have made observations on the heating effect of corn upon ducks and geese. Using the values obtained by direct calorimetry, the results may be summarized as shown in Table 1.

TABLE 1.—*The heating effect of corn on ducks and geese, as summarized from the experiments of Hári, and of Hári and Kriwuscha*

Bird	Body weight	Corn consumed	Heat production per day	Estimated basal heat production	Heating effect of corn	
					Observed	Calculated to 100 grams of corn
	Grams	*Grams*	*Calories*	*Calories*	*Calories*	*Calories*
Goose A	3,541	50	196	175	21	42
	3,499	100	274	172	102	102
	2,505	100	225	138	87	87
Goose B	3,126	50	241	153	88	176
	3,126	100	233	152	81	81
Duck A	967	50	127	90	37	74
Duck B	860	50	111	69	42	84

It is evident that the results obtained, calculated to 100 grams of corn, exhibited a rather wide variability, precluding any satisfactory estimate of a representative average.

In the same investigations the gross energy of food and solid and liquid excreta was determined, permitting an estimation of the metabolizable energy of corn for geese and ducks. The data covering metabolizable energy are summarized in Table 2.

TABLE 2.—*Metabolizable energy of corn for geese and ducks, as summarized from the experiments of Hári, and of Hári and Kriwuscha*

Bird	Body weight	Corn consumed	Gross energy of corn	Gross energy of excreta	Metabolizable energy of corn in—	
					Total calories	Percentage of gross energy
	Grams	*Grams*	*Calories*	*Calories*		
Goose A	3,541	50	202	38	164	81
	3,499	100	403	68	335	83
	2,505	100	403	80	323	80
Goose B	3,126	50	202	38	164	81
	3,126	100	403	65	338	84
Duck A	967	50	195	78	117	60
Duck B	860	50	195	75	120	62

For geese 82 per cent of the gross energy of corn appears to be metabolizable, while for ducks the much lower average of 61 per cent was obtained on two birds.

THE METABOLIZABLE ENERGY IN CORN

The first step in the present investigation was the determination of the metabolizable energy of corn. Results were obtained for 10 hens, 8 of which were Rhode Island Reds and 2 were White Plymouth Rocks. After several days' feeding of a constant amount of ground corn, the preliminary period being generally more than 10 days in length, the hens were placed in small cages, wire-mesh floors and movable pans being placed underneath for the collection of the excreta. The collection periods were seven days in length. The complete data resulting from this test are given in Table 3.

TABLE 3.—*Metabolizable energy of corn for hens*

Bird No.[a]	Average body weight	Average daily food	Daily energy intake	Energy of excreta per day	Metabolizable energy		
					Per day	Per 100 grams of corn	In terms of gross energy
	Grams	*Grams*	*Calories*	*Calories*	*Calories*	*Calories*	*Per cent*
129	2,126	44	181	28	153	348	84
130	1,610	44	181	31	150	341	83
132	1,407	42	172	30	142	338	83
135	1,732	44	181	31	150	341	83
74	2,144	50	196	32	164	328	84
129a	2,269	50	196	34	162	324	83
85	2,243	75	294	55	239	319	81
151	2,531	75	294	50	244	325	83
1		50	201	35	166	332	83
2	2,810	50	203	43	160	320	79

[a] Birds Nos. 1 and 2 were White Plymouth Rocks; the others were Rhode Island Reds.

The metabolizable energy of corn is taken as the difference between its gross-energy content and the gross energy contained in the excreta resulting from its digestion and assimilation. No correction has been made for the nitrogen balances of the birds. Such a correction is always small and can not be made with any great degree of accuracy, particularly with birds. It is also assumed that no appreciable amount of combustible gases is produced in the digestive tract of the chicken. The gross energy of the corn and excreta was determined in the Parr oxygen bomb calorimeter.

The percentage of the gross energy of corn that was metabolizable proved to be very constant, averaging 83.

All of the samples of corn used in these experiments were, unfortunately, not analyzed. However, that used for hens Nos. 74, 129a, 85, and 151 was found to contain 89.5 per cent dry matter, 3.49 per cent fat, and 1.56 per cent nitrogen, and to possess a gross-energy content of 3.917 calories per gram. The small variation in the composition of the samples of corn obtained for experimental work in this laboratory could hardly be considered as exerting any influence upon the percentage of its gross energy that would be metabolizable.

THE BASAL METABOLISM OF MATURE CHICKENS

Having determined the fraction of the gross energy of corn that is metabolizable, the next step in the investigation was to determine the basal heat production of mature chickens and the increase in heat production due to the consumption of corn. This heat increment represents a fraction of the metabolizable energy of corn that is inevitably wasted as heat. It is, therefore, just as surely an energy loss as the energy in the indigestible portion of the corn contained in the feces or that in the unoxidizable portion contained in the urine. Its determination is essential in arriving at a value for the net-energy content of corn.

The determination of heat production evidently requires the use of an animal calorimeter or respiration apparatus. In all of their calorimetric work on chickens the writers have used the gravimetric respiration apparatus of Haldane (*3*), as described and pictured in a previous publication from this laboratory (*10*). The writers have found that the method is simple of operation when its requirements have been carefully studied, economical of time, readily checked for possible errors, and capable of yielding consistent and accurate results except possibly for short observational periods. In the present investigation the observational periods were generally 22 to 23 hours in length. The weighing of the bottles and the feeding and watering of the chickens occupied the remaining one or two hours of the day. The rate of ventilation was approximately two liters per minute.

The heat production has been computed on the assumption that the total respiratory quotient is nonprotein. This method of calculation does not consider protein metabolism. However, from the data of Zuntz and Schumburg (*7, p. 62; 12*), relating to the calorific value of a liter of oxygen used in the combustion of the different nutrients, it may be computed that the maximum error in the total heat production figures resulting from this simplification is 6.58 per cent, and that the usual error would be less than 2 per cent.[4]

Total respiratory quotient	Maximum percentage of protein metabolism possible	Calorific value of a liter of O_2	Maximum error in assuming 100 per cent non-protein metabolism
0.75	44.65	4.594	3.06
.80	100.00	4.485	6.58
.85	73.12	4.624	4.92
.90	47.30	4.765	3.23
.95	22.97	4.906	1.58

Thus, if the total respiratory quotient is 0 85 and the protein metabolism is at its maximum (73.12 per cent of total calories), the error in neglecting the protein metabolism entirely would be less than 5 per cent. Under the much more usual conditions where the protein metabolism never exceeds 25 per cent of the total the error for all respiration quotients would be less than 1.5 per cent.

In the use of this or any other calorimetric method the temperature in the animal chamber must be under observation and should preferably be under control. During the daytime this could be

[4] Taking the calorific values of a liter of oxygen used in the combustion of protein, fat, and carbohydrate to be 4.485 calories, 4.686 calories, and 5 047 calories, respectively, the following is true:

accomplished readily. In view of the fact that the average critical temperature for Rhode Island Red hens is 62° (*10*), particular care was taken in this study that the temperature of the laboratory did not fall below 70° F. At night, however, control was not so effective, and occasionally the temperature fell considerably below 70°. A maximum and minimum thermometer was kept in the animal chamber in such position that its glass parts did not come in contact with the bird. The records read from this thermometer have been reported with all experiments.

The muscular activity of the chickens was also under continuous observation. The respiration chamber was suspended from a spring of such size that any considerable movement of the bird would result in an up-and-down movement of the chamber. This was recorded by a homemade "work adder" which revolved in only one direction. The record was, of course, purely of relative significance and served to assure the comparability of experimental periods as far as concerned the activity of the birds under observation. The animal chamber was not sufficiently large to permit of much movement, and the absolute darkness prevailing inside also tended to discourage much activity in the chicken. The work records have not been reported since (1) they indicate in general no great disproportion in activity among comparable experimental periods, (2) in the few cases in which a disproportionately large record was obtained the heat production of the bird was not out of line with that of comparable periods, and (3) the record has no definite or absolute significance with regard to the amount of movement within the chamber.

At the beginning it was considered necessary to determine how soon after feeding the heat production of the chicken returns to the basal level. From a large number of observations it was evident that, although the basal level was often reached after a fasting period of 24 hours, a fast of 48 hours was required before the basal level was reached in all cases. As illustrative of this finding, the data given in Table 4 are presented.

TABLE 4.—*Heat production of hens following the feeding of 75 grams of corn*

		Hen 2353				Hen 2001				Hen 200		
Day	Weight	Minimum and maximum temperature of chamber	Respiratory quotient	Heat production	Weight	Minimum and maximum temperature of chamber	Respiratory quotient	Heat production	Weight	Minimum and maximum temperature of chamber	Respiratory quotient	Heat production
	Grams			*Calories*	*Grams*			*Calories*	*Grams*			*Calories*
1____	2,200	90–91	0.89	212	3,667	83–90	0.92	195	2,530	85–88	0.91	166
2____	2,133	81–84	.94	153	3,533	82–90	.80	156	2,385	82–87	.79	ª 144
3____	2,047	85–90	.70	149	3,505	75–80	.69	151	2,287	80–93	.71	115
4____	1,942	85–94	.72	ª 154	3,463	70–86	.69	154	2,262	80–86	.71	110
5____	1,897	89–95	.74	ᵇ 151	3,415	85–89	.70	134	2,230	77–87	.70	106

ª Hen laid an egg weighing 52 gm. in respiration chamber.
ᵇ Eggshell found in respiration chamber. Apparently the hen had eaten contents.

TABLE 5.—*Basal heat production of mature chickens*

Bird No.	Date	Body weight	Body surface	Mini- mum and maxi- mum tempera- ture of chamber	Total	Per kilo- gram of body weight	Per square meter of body surface	Respira- tory quotient
Hens:		*Grams*	*Sq. cm.*		*Calories*	*Calories*	*Calories*	
E605	Nov. 9, 1925	1,925	1,551	63-70	126	65.5	812	0.70
	Nov. 17, 1925	1,880	1,533	62-80	132	70.2	861	.73
	Nov. 20, 1925	1,820	1,509	72-82	119	65.4	789	.71
	Dec. 4, 1925	1,715	1,464	72-82	100	58.3	683	.70
	Dec. 11, 1925	1,675	1,447	74-82	101	60.3	698	.70
	Dec. 14, 1925	1,630	1,428	72-78	88	54.0	616	.75
542	Sept. 28, 1925	1,790	1,496	70-82	109	60.9	729	.72
	Oct. 7, 1925	1,770	1,488	65-73	106	59.9	712	.71
	Oct. 10, 1925	1,740	1,475	63-71	102	58.6	692	.72
	Oct. 15, 1925	1,705	1,460	64-70	91	53.4	623	.71
	Oct. 18, 1925	1,645	1,434	54-62	90	54.7	628	.70
	Nov. 2, 1925	1,880	1,533	70-78	89	47.3	581	.73
	Nov. 5, 1925	1,880	1,533	71-76	91	48.4	594	.73
158 [a]	Jan. 29, 1923	1,657	1,379	67-75	94	56.7	682	.70
	Feb. 1, 1923	1,630	1,359	74-78	81	49.8	595	.80
132 [a]	Feb. 5, 1923	1,520	1,302	66-72	90	59.2	691	.70
	Feb. 8, 1923	1,525	1,304	72-80	82	53.8	629	.87
200	June 17, 1925	2,287	1,666	80-93	115	50.3	690	.71
	June 29, 1925	2,300	1,670	66-84	110	47.8	659	.71
	July 2, 1925	2,265	1,657	82-99	109	48.1	658	.71
	Sept. 7, 1925	1,990	1,554	80-89	103	51.8	663	.72
	Sept. 17, 1925	2,010	1,561	73-84	103	51.2	660	.70
	Sept. 20, 1925	1,990	1,554	69-79	92	46.2	592	.73
	Sept. 27, 1925	1,955	1,540	73-77	89	45.5	578	.72
	Oct. 11, 1925	1,895	1,516	62-66	102	53.8	673	.70
	Oct. 14, 1925	1,825	1,488	64-66	99	54.3	665	.65
A552	July 6, 1925	2,055	1,603	82-94	119	57.9	742	.70
	July 9, 9125	2,027	1,592	84-86	114	56.2	716	.72
2429 [a]	July 20, 1925	1,860	1,490	78-82	113	60 8	758	.70
	July 23, 1925	1,809	1,462	69-81	112	61.9	766	.71
546	Oct. 19, 1925	2,050	1,577	60-70	139	67.8	881	.71
	Oct. 25, 1925	2,020	1,565	68-74	120	59.4	767	.71
	Oct. 28, 1925	1,965	1,544	72-76	113	57.5	732	.72
	Nov. 3, 1925	1,900	1,518	67-75	110	57.9	725	.70
	Nov. 6, 1925	1,860	1,502	72-78	106	57.0	706	.70
35	Nov. 16, 1925	2,140	1,645	65-74	103	48.1	626	.71
	Nov. 23, 1925	2,115	1,636	58-72	102	48.2	623	.70
	Nov. 26, 1925	2,090	1,626	75-82	102	48.8	627	.72
	Dec. 4, 1925	2,100	1,632	74-84	85	40.5	520	.70
2420	Dec. 4, 1924	2,578	2,116	78-85	113	43.8	534	.72
2058	Dec. 11, 1924	2,195	1,759	80-92	122	55.6	694	.72
0	May 20, 1925	1,983	1,885	72-78	116	58.5	615	.69
2353	June 3, 1925	2,047	1,915	85-90	149	[b](72.8)	[b](778)	.70
2001	June 10, 1925	3,505	2,632	75-80	151	43.1	574	.69
Cocks:								
443 [a]	Aug. 25, 1924	2,750	1,933	78-88	148	53.8	766	.72
	Aug. 28, 1924	2,700	1,910	80-86	138	51.1	723	.69
458 [a]	Sept. 8, 1924	2,900	2,003	70-74	166	57.2	829	.70
	Sept. 11, 1924	2,800	1,957	72-82	154	55.0	787	.71
2284 [a]	Sept. 15, 1924	3,600	2,314	68-80	154	42.8	666	.72
	Sept. 18, 1924	3,450	2,249	68-76	166	48.1	738	.71
225 [a]	Sept. 22, 1924	3,235	2,155	68-82	191	59.0	886	.73
	Sept. 25, 1924	3,040	2,067	74-84	162	53.3	784	.72
161 [a]	Sept. 22, 1924	3,120	2,103	70-94	182	58.3	865	.71
	Sept. 25, 1924	2,945	2,024	73-93	173	58.7	855	.71
180 [a]	Aug. 18, 1924	2,330	1,731	73-84	127	54.5	734	.68
	Aug. 21, 1924	2,275	1,704	80-90	114	50.1	669	.72
552 [a]	Aug. 25, 1924	2,475	1,802	78-91	138	55.8	766	.73
	Aug. 28, 1924	2,410	1,771	72-88	131	54.4	739	.71

[a] The surface area of these birds was estimated by the Meeh formula (*9*), taking $k=9.85$. The surface area of all other birds was estimated from the weight and the rump-to-shoulder distance, according to the formula

$$S = 5.86 \ W^{0.5} \ L^{0.6}$$

of Mitchell, Card, and Hamilton (*11, p. 84*). Although this formula was obtained from White Plymouth Rock birds, it was shown to apply also to Rhode Island Red hens.
[b] Obtained during a period of egg production.

On the first experimental day these three Rhode Island Red hens were given a feeding of 75 gm. of ground corn before being placed in the respiration chamber; the heat production was then determined on five successive days in observational periods of 7 to 8 hours, the results being expressed on the 24-hour basis. In the case of hen 2353 the basal level was apparently established in 24 hours, but the fact that she was producing eggs possibly kept her heat production at a high level on the third and fourth days. The basal level was not reached with hen 200 and, less certainly with hen 2001, until they had fasted 48 hours. Many other experiments to be cited later support the conclusion that a hen can not be considered to be in the postabsorptive state until 48 hours have elapsed since the last feeding.

Before considering the experiments dealing with the heat increment due to feeding it is interesting to consider the results obtained on the basal heat production of Rhode Island Red chickens. Such data, collected from the feeding experiments, are reported in Table 5. The values obtained refer to the heat production after a 48-hour fast, observed in periods of 19 to 23 hours and expressed on the 24-hour basis. The results have also been expressed in calories per day per kilogram of body weight and per square meter of body surface (skin area).

Where more than one basal metabolism determination has been made upon a chicken the results have been arranged in the order in which they were obtained. It will be noted that in general a decrease in basal metabolism occurs during the course of a series of observations, particularly well shown with hens 542, 546, and E605. This decrease is probably comparable to that observed by Lusk and Du Bois (8) in dogs during a series of laboratory experiments and seems to be due to confinement and the resulting lowering of vitality. While there may be some suspicion that some of the results obtained are larger than the actual basal values because of exposure for short periods at night to environmental temperatures below the critical, the large majority of the heat productions do not appear to be complicated by this factor.

A number of other basal metabolism data on Rhode Island Red chickens are available from studies in which, for one reason or another, the heating effect of corn was not satisfactorily determined. The majority of these results were obtained at a time when the method of studying the effect of feeds on metabolism was being developed, and represent either initial determinations or, as in most cases, determinations after a three-day experimental period. They have been assembled in Table 6.

The homogeneity of the results given in Tables 5 and 6 is somewhat disturbed by the fact that the basal metabolism of the chickens in general decreased with the time spent on experiment. Hence, the average for a hen kept on experiment for a long period of time would not be comparable to that of a hen on experiment for a short period of time. A more significant average would, therefore, be obtained from the results secured in the first metabolism experiments on the birds, representing, as nearly as possible, their basal metabolism at the time of removal from the poultry farm.

TABLE 6.—*Basal heat production of a group of Rhode Island Red chickens*

Bird No.	Date	Body weight	Body surface [a]	Minimum and maximum temperature of chamber	Basal heat production per day			Respiratory quotient
					Total	Per kilogram of body weight	Per square meter of body surface	
Hens:		*Grams*	*Sq. cm.*		*Calories*	*Calories*	*Calories*	
230	Oct. 23, 1924	1,937	1,531	74–92	114	58.9	745	0.72
2217	Oct. 9, 1924	2,720	1,919	78–88	128	47.1	667	.72
2089	Oct. 16, 1924	2,490	1,810	74–84	127	51.0	702	.71
137	____do_____	2,330	1,731	70–80	117	50.2	676	.71
91	Oct. 23, 1924	2,025	1,577	70–89	116	57.3	736	.76
2429	Oct. 30, 1924	1,940	1,532	77–87	117	60.3	764	.74
2076	____do_____	2,132	1,632	76–86	124	58.2	760	.73
2053	Nov. 6, 1924	2,300	1,716	76–86	129	56.1	752	.72
2013	Nov. 13, 1924	2,295	1,714	76–84	130	56.6	759	.72
2160	____do_____	2,242	1,687	76–86	113	50.4	670	.72
2210	Nov. 26, 1924	1,845	1,482	68–85	84	45.5	567	.87
2353	____do_____	1,705	1,406	70–93	106	62.2	754	.72
200	Dec. 4, 1924	2,445	1,788	72–90	115	47.0	643	.74
2009	Dec. 11, 1924	2,405	1,768	74–90	119	49.5	673	.71
127	Jan. 26, 1923	2,138	1,635	60–78	101	47.2	618	.73
73	Feb. 12, 1923	2,575	1,851	66–82	160	62.1	864	.72
Cocks:								
174	Sept. 1, 1924	2,475	1,802	72–84	150	60.6	832	.67
	Sept. 4, 1924	2,400	1,766	74–82	124	51.7	702	.72
816	Sept. 1, 1924	2,812	1,962	76–88	187	66.5	953	.72
	Sept. 4, 1924	2,700	1,910	73–78	164	60.7	859	.71
328	Sept. 11, 1924	2,820	1,966	71–77	151	53.5	768	.72
316	Sept. 15, 1924	3,290	2,179	66–90	228	69.3	1,046	.70
	Sept. 18, 1924	3,085	2,087	68–80	196	63.5	939	.69
524	Oct. 2, 1924	3,125	2,105	68–90	178	57.0	846	.72
541	____do_____	2,740	1,929	70–86	141	51.5	731	.73
721	July 21, 1924	2,475	1,803	76–88	146	59.0	810	.73
	July 24, 1924	2,400	1,766	81–82	112	46.7	634	.73
398	July 31, 1924	2,650	1,886	80–88	131	49.4	695	.68
474	Aug. 7, 1924	3,000	2,049	76–88	148	49.3	722	.73
524	Aug. 11, 1924	3,030	2,063	77–84	160	52.8	776	.68
	Aug. 14, 1924	2,970	2,035	72–80	155	52.2	762	.70
427	Aug. 11, 1924	3,010	2,054	72–78	158	52.5	769	.69
	Aug. 14, 1924	2,960	2,031	72–82	147	49.7	724	.71
446	Aug. 18, 1924	3,185	2,132	72–82	180	56.5	844	.68
	Aug. 21, 1924	2,900	2,003	82–90	186	64.2	929	.71

[a] The surface area of these birds was estimated by the Meeh formula (*9*), taking $k=9.85$.

Considering only the first successful determinations made upon the birds, preceded at most by three days' confinement in the respiration apparatus, the average basal heat production for the 28 hens was 54.9 calories per day per kilogram of body weight, or 703 calories per day per square meter of body surface. For the 19 cocks the averages were 55.7 calories and 806 calories, respectively. These results indicate a distinct sex difference in basal metabolism, in agreement with work upon other animals. The standard deviations of the individual determinations were 6.64 calories per kilogram body weight, and 81.5 calories per square meter body surface for the hens and 5.76 calories and 88.2 calories for the cocks. The coefficients of variation were 12.1 per cent on the body weight and 11.6 per cent on the body surface in the case of the hens and 10.3 per cent on the body weight and 10.9 per cent on the body surface in the case of the cocks. These coefficients of variation may be compared with those computed by Harris and Benedict (*6*) for human subjects. For men the coefficients found were 9.36 for the basal metabolism referred to weight and 8.05 for the basal metabolism referred to surface. For women the coefficients were 14.14 and 9.17, respectively.

The average respiratory quotient in the experiments reported in Table 5 was 0.713, and in the experiments reported in Table 6, 0.719. Evidently the metabolism of the chicken in the period from the forty-eighth to the seventieth hours after feeding is almost entirely at the expense of fat. While a small minority of the individual quotients were below the respiratory quotient for fat, such deficits are quite probably due to slight errors in the oxygen determinations and are so considered in the calculation of the heat production. The writers did not obtain the consistently low values for the quotient during fast reported by Gerhartz (*2*) in experiments on hens. The results of the broader but contradictory experience of the writers, as well as the inherent improbability of the general occurrence of conditions that would force the respiratory quotient to 0.65, 0.60, or lower values in short periods of fast, may well indicate that these low values are due to technical errors.

The basal heat production of hens just considered refers to the non-laying hen. The only comparable values that have been found in the literature are those of Gerhartz (*2*) upon two hens. The basal heat production in three experiments for one of these hens, in a period of sexual inactivity, 13 to 23 hours after feeding, was found to be 584, 681, and 676 calories daily per square meter of body surface as computed by the Meeh formula (*9*) where $k = 9.49$. In the brooding period this hen gave values of 785 and 779 calories and another hen values of 917 and 795 calories. All of these values are quite within the range of variation found in the writers' experiments. Hári (*4*) has reported average basal heat production values of 698 and 1,036 calories per day per square meter of body surface for two geese; and Hári and Kriwuscha (*5*) values of 735 and 901 calories for two ducks.

According to Gerhartz, the hen producing eggs has a much larger basal metabolism than the nonlaying hen, a difference (increase) of 44 per cent being noted in one hen. Although the writers obtained their data largely from nonlaying hens, occasionally an egg was laid during an experimental observation. In Table 4 the egg production of hen 2353 apparently increased her basal heat production, since at the end of 48 hours of fast it amounted to 72.8 calories per day per kilogram, or 778 calories per day per square meter of body surface. (See Table 5.) The apparently large heating effect of 75 gm. of corn with hen 200 (Table 4) is also plausibly explained by the production of an egg on the second day of the experiment. On the other hand, in experiment 21 on hen A552 (see Table 9) the production of an egg on the seventh day of the experiment did not appear to disturb the level of heat production, nor was the estimated heating effect of the corn excessive, even though the basal value, 716 calories per day per square meter of surface, was near the average for all of the hens. The subject evidently requires further investigation, particularly since there is no obvious reason why egg production in the hen, any more than milk production in the cow, should increase the basal metabolism and the maintenance requirement.

THE HEATING EFFECT OF CORN

The corn used in the respiration experiments to determine the heat increment due to feeding was analyzed from time to time, with the results shown in Table 7.

TABLE 7.—*Chemical composition of corn used in respiration experiments*

| Sample No. | Percentage of— | | | | | | Gross energy (calories per gram) |
	Dry matter	Crude protein	Nitro-gen-free extract	Crude fiber	Fat	Ash	
1	91.2	10.0	73.9	2.68	3.27	1.38	4.16
2	92.4	8.3	76.1	2.99	3.70	1.35	4.10
3	92.1	9.7	74.1	2.86	3.99	1.39	4.13
4	87.9	9.0	73.2	2.18	2.19	1.33	3.78
5	89.9	8.8	73.5	2.69	3.74	1.19	3.99
Average	90.7	9.16	74.2	2.68	3.38	1.33	4.02

In view of the small variation among these analyses, particularly with reference to dry matter, it was not considered necessary to relate definite samples with definite experiments. The respiration experiments to be described may therefore be considered to relate to corn containing 91 per cent of dry matter and 4.02 calories of gross energy per gram.

In the first series of experiments on the heating effect of corn the plan was to determine the basal metabolism of the chicken after a 48-hour fast, to give 50 or 75 gm. of ground corn in one feeding at the beginning of the second experimental day, and to determine the 24-hour heat production on the day of feeding and on the two following days. The last day's result, obtained after 48 hours of fast, was considered to represent the basal metabolism. It was, in general, lower than the result of the first basal period. The increase in heat production due to the feeding of corn was computed by assuming that the decrease in basal metabolism, whenever such occurred, was linear. Where the variation in the weight of the bird was considerable this factor also was considered in estimating the basal metabolism on the day of feeding and on the following day. The total heating effect per 100 gm. of corn was then computed as the final result in each experiment. Before each day's experiment the chicken was given 50 c. c. of water by pipette. The results of 20 experiments of this type upon 15 Rhode Island Red chickens are summarized in Table 8.

TABLE 8.—*Heating effect of corn on mature chickens (one feeding)*

Bird No.	Day of test	Weight of bird	Treatment	Minimum and maximum temperature of chamber	CO_2 given off per 24 hours	O_2 per 24 hours	Respiratory quotient	Heat production per 24 hours	Estimated basal heat production	Effect of corn Observed	Effect of corn Calculated to 100 grams of corn
		Gms.			*Gms.*	*Gms.*		*Calories*	*Calories*	*Calories*	*Calories*
546	1	2,020	Fasted 48 hours	68–74	35.9	36.5	0.71	120			
	2	2,030	Fed 75 gm. corn	62–70	60.7	42.6	1.03	151	118	33	
	3	2,020	Fasted	70–74	38.7	37.6	.75	125	116	9	56
	4	1,965	....do	72–76	34.0	34.4	.72	113			
546	1	1,900	Fasted 48 hours	67–75	32.3	33.4	.70	110			
	2	1,895	Fed 50 gm. corn	68–78	49.8	37.3	.97	131	109	22	
	3	1,895	Fasted	70–74	34.8	34.1	.74	113	108	5	54
	4	1,860	....do	72–78	31.1	32.2	.70	106			
E605	1	1,880	Fasted 48 hours	62–80	39.9	39.9	.73	132			
	2	1,880	Fed 75 gm. corn	70–78	59.6	43.6	.99	154	128	26	
	3	1,875	Fasted	72–78	39.6	37.7	.76	126	123	3	40
	4	1,820	....do	72–82	35.4	36.4	.71	119			
E605	1	1,675	Fasted 48 hours	74–82	29.5	30.8	.70	101			
	2	1,675	Fed 50 gm. corn	72–82	42.3	31.7	.97	111	97	14	
	3	1,670	Fasted	73–85	29.8	27.7	.78	93	92	1	30
	4	1,630	....do	72–78	27.3	26.6	.75	88			
542	1	1,705	Fasted 48 hours	64–70	27.1	27.8	.71	91			
	2	1,710	Fed 50 gm. corn	62–65	41.0	30.7	.97	108	91	17	
	3	1,690	Fasted	58–66	29.8	28.0	.77	94	90	4	42
	4	1,645	....do	54–62	26.2	27.3	.70	90			
542	1	1,880	Fasted 48 hours	70–78	26.9	26.9	.73	89			
	2	1,930	Fed 75 gm. corn	69–79	47.7	34.9	.99	123	90	33	
	3	1,945	Fasted	70–80	38.2	31.6	.88	108	90	18	68
	4	1,880	....do	71–76	27.9	27.6	.73	91			
158	1	1,657	Fasted 48 hours	67–75	27.2	28.6	.70	94			
	2	1,686	Fed 75 gm. corn	66–80	42.4	31.5	.98	111	90	21	
	3	1,700	Fasted	74–82	40.1	28.4	1.03	100	86	14	47
	4	1,630	....do	74–78	26.6	24.2	.80	81			
132	1	1,521	Fasted 50 hours	66–72	26.2	27.4	.70	90			
	2	1,553	Fed 75 gm. corn	72–76	41.2	31.0	.96	109	87	22	
	3	1,572	Fasted		38.5	29.4	.95	103	84	19	55
	4	1,525	....do	72–80	28.9	24.1	.87	82			
200	1	2,300	Fasted 46 hours	66–84	31.5	32.1	.71	110			
	2	2,317	Fed 75 gm. corn	74–86	50.2	38.3	.95	134	110	24	
	3	2,330	Fasted	78–88	43.0	36.3	.86	124	110	14	51
	4	2,265	....do	82–99	32.5	33.2	.71	109			
A552	1	2,055	Fasted 46 hours	82–94	34.9	36.3	.70	119			
	2	2,063	Fed 75 gm. corn	82–92	50.4	37.8	.97	132	117	15	
	3	2,065	Fasted	82–88	43.8	38.2	.83	130	116	14	40
	4	2,027	....do	84–86	34.3	34.5	.72	114			
2429	1	1,860	Fasted 48 hours	78–82	33.1	34.4	.70	113			
	2	1,857	Fed 75 gm. corn	74–88	48.1	37.1	.94	129	112	17	
	3	1,864	Fasted	68–83	43.1	36.7	.86	125	112	13	40
	4	1,809	....do	69–81	33.3	34.0	.71	112			
35	1	2,115	Fasted 48 hours	58–72	30.1	31.1	.70	102			
	2	2,140	Fed 75 gm. corn	76–80	52.1	36.4	1.04	129	102	27	
	3	2,135	Fasted	74–79	36.6	31.5	.84	107	102	5	43
	4	2,090	....do	75–82	30.6	30.9	.72	102			
200	1	2,010	Fasted 48 hours	73–84	30.4	31.5	.70	103			
	2	2,035	Fed 75 gm. corn	80–87	51.4	36.2	1.03	129	99	30	
	3	2,040	Fasted	80–88	37.9	32.0	.86	109	95	14	59
	4	1,990	....do	69–79	28.1	28.0	.73	92			
443	1	2,750	Fasted 48 hours	78–88	44.5	45.1	.72	148			
	2	2,720	Fed 75 gm. corn	80–88	62.0	50.2	.90	173	145	28	
	3	2,760	Fasted	80–90	48.1	47.5	.74	157	142	15	57
	4	2,700	....do	80–86	40.0	42.0	.69	138			
552	1	2,475	Fasted 48 hours	78–91	42.2	41.7	.73	138			
	2	2,445	Fed 75 gm. corn	82–90	64.2	52.3	.89	180	136	44	
	3	2,475	Fasted	77–93	42.4	42.2	.73	139	134	5	65
	4	2,410	....do	72–88	39.0	39.8	.71	131			
458	1	2,900	Fasted 48 hours	70–74	48.8	50.7	.70	166			
	2	2,900	Fed 75 gm. corn	72–80	69.9	55.0	.92	191	162	29	
	3	2,845	Fasted	70–82	49.4	49.4	.72	163	158	5	45
	4	2,800	....do	72–82	45.8	46.9	.71	154			
2,284	1	3,600	Fasted 48 hours	68–80	46.0	46.8	.72	154			
	2	3,515	Fed 75 gm. corn	72–84	70.3	54.4	.94	189	158	31	
	3	3,520	Fasted	66–80	53.8	51.8	.75	172	162	10	55
	4	3,450	....do	68–76	49.5	50.5	.71	166			

TABLE 8.—*Heating effect of corn on mature chickens (one feeding)*—Continued

Experiment No	Bird No.	Day of test	Weight of bird	Treatment	Minimum and maximum temperature of chamber	CO₂ given off per 24 hours	O₂ per 24 hours	Respiratory quotient	Heat production per 24 hours	Estimated basal heat production	Effect of corn Observed	Effect of corn Calculated to 100 grams of corn
			Gms.			*Gms.*	*Gms.*		*Calories*	*Calories*	*Calories*	*Calories*
18	225	1	3,235	Fasted 48 hours	68–82	57.9	57 9	0.73	191			
		2	3,150	Fed 75 gm. corn	70–80	74.8	61.4	.89	211	181	30	
		3	3,100	Fasted	71–81	62.2	55.0	.82	186	171	15	60
		4	3,040	do	74–84	48.6	49.2	.72	162			
19	161	1	3,120	Fasted 48 hours	70–94	54.4	55.4	.71	182			
		2	3,080	Fed 75 gm. corn	66–78	76 1	65.6	.84	223	179	44	
		3	3,000	Fasted	70–76	62.0	57.4	.78	192	176	16	(93)
		4	2,945	do	73–93	51.8	52.7	.71	173			
20	180	1	2,330	Fasted 48 hours	73–84	36.2	38.6	.68	127			
		2	2,275	Fed 75 gm. corn	76–82	57.1	43.2	.96	151	123	28	
		3	2,275	Fasted	78–86	41.6	39.4	.77	131	119	12	53
		4	2,275	do	80–90	34.3	34.5	.72	114			

Another method of attacking the same problem is to compare the level of heat production established by the continuous feeding of a constant amount of corn with the basal heat production. In this type of experiment the basal metabolism was determined after 48 hours of fast, after which the chickens were given 50 or 75 gm. of ground corn and an adequate and constant amount of water early each morning. The heat production was determined daily until, for several consecutive days, a level appeared to have been established, following which food was withdrawn and a basal determination again made after a fast of 48 hours. The heating effect of the corn fed was then taken as the difference between the average heat production on feed and the average estimated basal heat production. Eleven experiments of this type upon five different Rhode Island Red hens are summarized in Table 9.

TABLE 9.—*Heating effect of corn on hens (continuous feeding)*

Experiment No.	Bird No.	Day of test	Weight of bird	Treatment	Minimum and maximum temperature of chamber	CO₂ given off per 24 hours	O₂ consumption per 24 hours	Respiratory quotient	Heat production per 24 hours	Average heat production on feed	Estimated basal heat production	Effect of corn Observed	Effect of corn Calculated to 100 grams
			Grams			*Gms.*	*Gms.*		*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
21	A552	1	2,027	Basal	84–86	34.3	34.5	0.72	114				
		5	2,210	Fed 75 gm. corn	77–91	57.2	42.7	.97	150				
		6	2,170	do	76–84	68.0	47.2	1.05	168				
		a 7	2,157	do	84–88	58.4	44.6	.95	156				
		8	2,135	do	81–97	68.3	45.5	1.09	163	159	122	37	50
22	546	1	2,050	Basal	60–70	41.4	42.3	.71	139				
		3	2,095	Fed 75 gm. corn	64–86	71.9	48.4	1.08	173				
		4	2,100	do	68–87	69.3	47.0	1.07	168				
		5	2,105	do	69–85	68.6	46.5	1.07	166	169	133	36	
		7	2,020	Basal	68–74	35.9	36.5	.71	120				

a Hen laid an egg (45 gm.) in respiration chamber.

TABLE 9.—*Heating effect of corn on hens (continuous feeding)*—Continued

Experiment No.	Bird No.	Day of test	Weight of bird	Treatment	Minimum and maximum temperature of chamber	CO2 given off per 24 hours	O2 consumption per 24 hours	Respiratory quotient	Heat production per 24 hours	Average heat production on feed	Estimated basal heat production	Effect of corn Observed	Effect of corn Calculated to 100 grams
			Grams			*Gms.*	*Gms.*		*Calories*	*Calories*	*Caes kilo-*	*Calories*	*Calories*
23	546	1	1,965	Basal	72-76	34.0	34.4	0.72	113				
		3	1,950	Fed 50 gm. corn	68-76	53.7	40.4	.96	142				
		4	1,950	....do	68-72	55.2	41.0	.98	144				
		5	1,955	....do	70-76	55.9	40.6	1.00	143	143	112	31	62
		7	1,900	Basal	67-75	32.3	33.5	.70	110				
24	E605	1	1,925	....do	63-70	37.2	38.3	.70	126				
		3	1,955	Fed 75 gm. corn	68-82	62.2	43.9	1.03	156				
		4	1,965	....do	76-79	63.3	44.0	1.04	157				
		5	1,975	....do	68-80	66.7	44.6	1.09	160				
		6	1,980	....do	74-78	64.2	44.0	1.06	157				
		7	1,975	....do	62-76	64.2	44.2	1.06	159	138	129	29	39
		9	1,880	Basal	62-80	39.9	39.9	.73	132				
25	E605	1	1,820	....do	72-82	35.4	36.4	.71	119				
		3	1,840	Fed 50 gm. corn	54-72	56.0	40.6	1.00	143				
		4	1,830	....do	54-77	51.7	40.0	.94	139				
		5	1,835	....do	74-98	51.3	37.9	.98	134	138	119	19	38
		6	1,855	....do	76-84	50.6	38.8	.95	135				
26	E605	1	1,715	Basal	72-82	29.3	30.5	.70	100				
		3	1,740	Fed 50 gm. corn	70-82	46.8	34.2	.99	121				
		4	1,755	....do	70-84	48.7	34.6	1.02	123				
		5	1,750	....do	76-84	47.5	34.6	1.00	122				
		6	1,745	....do	74-94	49.6	35.6	1.01	126	123	101	22	44
		8	1,675	Basal	74-82	29.5	30.8	.70	101				
27	542	1	1,790	....do	70-82	32.7	33.1	.72	109				
		6	1,835	Fed 75 gm. corn	68-75	59.5	40.1	1.00	142				
		7	1,830,	....do	65-75	60.9	39.3	1.13	141				
		8	1,830	....do	68-76	59.9	40.1	1.09	143	142	108	34	45
		10	1,770	Basal	65-73	31.4	32.3	.71	106				
28	542	1	1,740	....do	63-71	30.6	30.9	.72	102				
		2	1,730	Fed 50 gm. corn	63-73	44.3	33.6	.96	118				
		3	1,735	....do	65-71	47.6	32.6	1.06	116				
		4	1,735	....do	68-70	46.9	33.0	1.03	117	117	96	21	42
		6	1,705	Basal	64-70	27.1	27.8	.71	91				
29	35	1	2,140	....do	65-74	30.5	31.4	.71	103				
		3	2,180	Fed 75 gm. corn	70-77	65.0	44.0	1.07	157				
		4	2,165	....do	70-78	66.8	46.2	1.05	165				
		5	2,165	....do	76-78	66.6	44.4	1.09	159				
		6	2,170	....do	70-76	65.7	44.8	1.06	159	160	105	55	73
		8	2.115	Basal	58-72	30.1	31.1	.70	102				
30	200	1	1,990	....do	80-89	31.0	31.4	.72	103				
		3	2,017	Fed 75 gm. corn	80-89	62.9	41.6	1.11	147				
		4	2,045	....do	80-86	63.4	41.6	1.11	149				
		5	2,070	....do	80-82	68.9	43.4	1.15	157				
		6	2,070	....do	74-78	67.9	46.4	1.06	(165)				
		7	2,080	....do	74-79	65.6	42.3	1.13	152				
		8	2,080	....do	73-79	62.3	40.9	1.11	147				
		9	2,080	....do	72-77	62.5	41.2	1.10	148	150	106	44	59
		11	2,010	Basal	73-84	30.4	31.5	.70	103				
31	200	1	1,990	....do	69-79	28.1	28.0	.73	92				
		2	1,985	Fed 50 gm. corn	65-72	45.5	35.6	.93	123				
		3	2,000	....do	65-70	54.2	38.2	1.03	(136)				
		4	2,000	....do	65-75	49.7	35.3	1.02	125				
		5	2,000	....do	62-76	49.9	35.6	1.02	126				
		6	2,000	....do	62-73	49.0	34.7	1.02	123	124	91	33	66
		8	1,955	Basal	73-77	26.6	27.0	.72	89				

In considering these two sets of data it is well at the start to determine how homogeneous they are, particularly with respect to (1) the type of experiment and (2) the amount of food given. Disregarding the exceptionally high result of experiment 19 in the first series of experiments, there were 16 involving a feeding of 75 gm. of corn and only 3 involving a feeding of 50 gm. The average heating

effect of corn per 100 gm. was in the first case 52 calories and in the second case 42 calories, the average for the entire series being 50 calories. Among the second series of experiments there were 6 involving a feeding of 75 gm. portions of corn and 5 involving a feeding of 50 gm. portions. The average heating effects were, respectively, 52 calories and 50 calories per 100 gm. of corn; and for the entire group 51 calories. From these averages it may be concluded, in the first place, that the two types of experiments gave essentially the same results. In the early experiments the heating effect extended over two days; animals fed 75 gm. of corn excreted on an average 70 per cent of the extra heat on the day of feeding and 30 per cent on the day following, while animals fed 50 gm. portions excreted on an average 85 per cent the first day and only 15 per cent on the day following. In the second series of experiments, therefore, the level of heat production established by continuous feeding must have exceeded that established by one feeding by exactly the "carry-over" effect of the day following one feeding.

In the second place, the above averages indicate that the smaller portion of corn *may* have had a slightly smaller heating effect per 100 gm. than the larger, but it is evident from an inspection of the tables that the data are too few and too variable to establish the significance of the average differences obtained.

Hence, the data may be considered and treated as a homogeneous series as far as these possible vitiating factors are concerned. It is true that where several tests were made upon the same bird there is some indication of rather distinct individual differences, as the summary in Table 10 shows.

TABLE 10.—*Difference among individual birds in the heating effect of corn per 100 gm.*

Bird No.	Experiment No.	Heat increment per 100 gm. of corn	Bird No.	Experiment No.	Heat increment per 100 gm. of corn
546	1	58	542	5	42
	2	54		6	68
	22	48		27	45
	23	62		28	42
E605	3	40	A552	10	40
	4	30		21	50
	24	39	200	9	51
	25	38		13	59
	26	44		30	58
				31	66
			35	12	43
				29	73

The results obtained with hen 200 are quite sharply distinguished from those obtained with hen E605. Hen 546, except for one of the four experiments, gave higher results than the average, and hen 542, except for one result, gave lower figures than the average. These differences are undoubtedly a source of heterogeneity. However, in view of the number of results obtained and the many other uncontrolled factors that were evidently operating in determining the estimated heating effect of corn, the effect of these differences is probably not serious.

The results of seven experiments upon cocks are summarized in Table 8. Omitting the anomalous result of experiment 19, the average heating effect of corn was 56 calories per 100 gm. For the 13 results on hens the average was 48 calories. The slight difference may be of significance in indicating a smaller heating effect for hens, but the data are too few and variable to permit a definite conclusion on this interesting point.

It is necessary to inquire whether the low minimum temperatures of the animal chamber prevailing at some period during the night in several of the experiments might not have operated in vitiating the estimated heat effect of corn on the metabolism of the chickens. The effect of temperatures below the critical would be to raise the heat production above the level that would otherwise prevail. For any low temperature the effect, if existent, would be more marked in the basal periods than in the feeding periods, since the critical temperature in the basal periods would be higher. Hence, where low temperatures exert such a vitiating effect the result would always be to depress the estimated heat increment below the actual. It is extremely doubtful whether on any of the feeding days the temperature in the chamber ever was less than the critical temperature of the fed bird.

It has been shown elsewhere by the writers (10) that the average critical temperature of the fasting Rhode Island Red hen is 62° F., though individual hens may show considerable variability in this respect. There were six experiments in which at least one of the two basal periods possessed a minimum temperature of 62° or below. The average estimated heat increment for these six experiments was 47, as compared with the average of 51 for the entire investigation. Among the six results is included the highest value, 73, obtained in this study, disregarding the anomalous result of experiment 19. The two lowest found, 30 and 38 (experiments 4 and 25), were obtained under temperature conditions above suspicion.

In this connection a further study of Table 10 is of interest. The lowest result for hen 546 is open to suspicion because of a minimum temperature of 60° F. in one of the basal periods, while for hen 542 the three low results may be considered suspicious for the same reason. However, for hen 35 no such explanation of the low value is plausible, while the relative constancy in results for hens E605 and 200 was obtained in spite of a wide variety of temperature conditions.

While it appears, therefore, that some of the low estimates of the heating effect of corn may have been occasioned in part by the prevalence during a fraction of the basal periods of temperatures below the critical, it may readily be shown that such temperature effects were not of serious moment. Eliminating the results of experiment 19 and of 6 experiments in which, for at least one of the basal periods, the minimum temperature was 62° or below, gives an average result for the remaining 24 experiments of 52 calories per 100 gm. of corn. If 8 experiments in which, for at least one of the basal periods, the minimum temperature was 65° or below are disregarded, the average result of the remaining 22 experiments is 52 calories per 100 gm. of corn. If the process of elimination is carried further to include all experiments in which the minimum temperature was 70° or below for at least one of the basal periods the average of the remaining 9 experiments becomes 48 calories.

The average heating effect of corn for all 30 experiments (excluding No. 19) is 50.7 ± 1.2 calories per 100 gm. of corn. The standard deviation of the individual determinations is 10.04 calories, and the coefficient of variation 19.8 per cent.

THE NET-ENERGY VALUE OF CORN FOR MATURE CHICKENS

Summing up the results of the investigation, it has been found that 83 per cent of the gross energy of ground corn is metabolizable by chickens and that the average heating effect of corn on cocks and nonlaying hens is 51 calories per 100 gm. The corn used in these investigations contained 402 calories per 100 gm. Its metabolizable energy would, therefore, be 334 calories and its net energy $334 - 51 = 283$ calories per 100 gm., or 1,285 calories per pound. Since the corn used in this investigation contained an average of 91 per cent of dry matter, the net-energy value of corn is 3,110 calories per kilogram of dry matter, or 1,412 calories per pound of dry matter. The latter figure may be compared with the value Armsby gives (*1, p. 660, 722*) for cattle, 926 calories per pound of dry matter, and his estimate for swine, i. e., 1,355 calories. Evidently the nonlaying hen ranks high in her ability to convert the energy of corn to her own uses.

SUMMARY

In experiments upon 10 chickens it has been found that 83 per cent of the gross energy of ground corn is metabolizable.

The average basal heat production of 28 nonlaying hens was found to be 54.9 calories per day per kilogram body weight and 703 calories per day per square meter body surface. For 19 mature cocks the average values found were 55.7 and 806 calories, respectively. These values may be considerably reduced during protracted confinement in the laboratory. The basal metabolism of hens is apparently raised considerably during periods of egg production, though this question requires much further study.

In 30 experiments upon 15 mature Rhode Island Red chickens an average heating effect of 50.7 ± 1.2 calories per 100 gm. of corn (91 per cent dry matter) has been obtained. After a single feeding of 75 gm. of ground corn an average of 70 per cent of the extra heat is eliminated during the first 24 hours and 30 per cent during the second 24 hours. For a single feeding of 50 gm. of corn 85 per cent of the extra heat is excreted in 24 hours and 15 per cent in the second 24-hour period. The chicken, therefore, can not be considered to be in the postabsorptive condition until more than 24 hours have elapsed since the last feeding. After 48 hours this condition may be considered to have been established. Some evidence was obtained indicating that the heating effect of corn is slightly greater with cocks than with hens.

The net-energy value of corn for mature chickens has been found to be 3,110 calories per kilogram of dry matter, or 1,412 calories per pound of dry matter.

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THE EFFECT OF AGE, SEX, AND CASTRATION ON THE BASAL HEAT PRODUCTION OF CHICKENS [1]

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INTRODUCTION

The basal heat production of an animal is its minimum energy expenditure. This factor determines the minimum requirement of food energy, and appears to be one of the most constant of biological measurements. When dividing an animal's energy expenditures into independent factors, the basal heat production is the factor upon which all others are imposed, and it becomes, therefore, the logical starting point in experimental investigations of this nature. Its intimate relation to the maintenance requirement of energy, of which it is the most important part, gives it a practical as well as a scientific significance.

A study of the basal heat production of chickens should start with the incubating egg if the developing embryo could be reduced to the fasting condition. The metabolism of the embryo within the egg involves the basal expenditure of energy and also whatever calorigenic effect the absorbed nutrient material may exert upon the embryonic tissue. It is an interesting fact, however, that the carbon dioxide production of the developing chick increases in proportion to its weight (*9, 10, 11, 20, 21*)[2] and that the heat produced, except possibly during the first few days, is essentially at the expense of fat.

During its life within the egg, the chick is cold-blooded (*35*), except for the few hours preceding hatching, when the transformation from the cold-blooded to the warm-blooded state occurs. In other words, during incubation the heat production of the embryo varies directly with its environmental temperature, responding to a fall in temperature with a fall in heat production, and vice versa. The writers have been able to confirm the results of Pembrey, Gordon, and Warren on this point.[3] However, during its first day or so of life, the

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[1] Received for publication Feb. 8, 1927; issued July, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 958.
[3] Two eggs showed the following responses to temperature changes, one on the 19th and one on the 20th day of incubation:

Day of incubation	Temperature of receptacle	CO₂ produced per 30 minutes	Day of incubation	Temperature of receptacle	CO₂ produced per 30 minutes
	°C.	Mgm.		°C.	Mgm.
Nineteenth	40	14.0	Twentieth	40	12.4
	40	15.2		40	13.8
	40	15.1		25	7.5
	25	7.6		40	14.5
	40	10.2		40	14.5
	40	13.8		40	14.8
	25	7.8		25	7.9

(945)

chick quickly assumes the characteristics of warm-bloodedness. It becomes less a creature of circumstance. Throughout a considerable temperature range, its metabolism is unaffected; its body temperature is maintained constant at varying environmental temperatures by physiological regulation of the emission of heat from its body. When this regulatory mechanism is strained to the utmost by decreasing temperatures, it is still able to maintain its body temperature by increasing its heat production, the direct opposite of its response intra ovum.

In its changing response to environmental temperatures previous and subsequent to hatching, the embryo is reminiscent of the hibernating animal. During hibernation, the response of an animal is typically cold blooded. It lacks the capacity of maintaining its body temperature constant, and its metabolism, in the prevailing cold weather, decreases, often to a mere fraction of that which characterizes its active existence. When environmental conditions, particularly with reference to temperature, become suitable, the animal wakes from hibernation, there is an outburst of metabolic activity and a rapid increase in heat production, and as a result the body temperature quickly rises to normal (*3, 17, 18, 31, 32, 33, 34, 36*).

A similar outburst of metabolic activity occurs in the hatching chick, although not of the same relative magnitude. Thus, the carbon dioxide production of an embryo on the nineteenth day of incubation was 29 mgm. per hour, and that of another embryo on the twentieth day was 28 mgm. per hour. On the other hand, a chick of the same breed 1 day old, when quiet and fasting, produced 68 mgm. of CO_2 per hour; another chick, 2 days old, produced 61 mgm. per hour, while a third chick, 3 days old, produced 61.4 mgm. Since the respiratory quotient of the incubating embryo is similar to that of the fasting chick, the rates of heat production of the two stand in the same ratio as the rates of production of carbon dioxide. The experimental observations of Pembrey, Gordon, and Warren (*35*) also indicate a marked increase in the metabolism of the chick at hatching.

The effect of age and sex upon the basal metabolism has not been studied in any great detail except with human subjects. In 1916, Du Bois (*15*) presented a curve showing the change in the basal metabolism per unit of body surface from birth to old age. During the first few weeks of life the metabolism of the infant was found to be very low (27 to 30 calories per square meter per hour); it then rose rapidly until at 1 year of age it was 50 per cent above the adult level. The maximum was reached in the "almost unexplored" period between 2 and 6 years of age (about 60 calories per square meter per hour), after which the metabolism fell rapidly to 20 years of age, and extremely gradually after that. The essential features of this curve have been confirmed by Benedict and Talbot (*6, 8*), and especially by the more recent data of Talbot (*37*). Mitchell and Carman (*27*) observed a very constant rate of basal metabolism in rats from 90 to 190 days of age, though at an earlier age (30 to 40 days) distinctly higher rates were noted. Essentially the same effect of age upon basal metabolism was observed in growing pigs by Deighton (*14*) and Wood (*38*).

It is generally recognized that females possess a lower rate of basal metabolism than males (*4, 7, 19*), and with human subjects, accord-

ing to Benedict (*6*, *7*), the sex difference appears at an early age, represented by a body weight of 10 kgm. In the human species, the difference in basal metabolism between the sexes is ordinarily rated at 5 to 7 per cent.

EXPERIMENTAL METHODS

In the writers' studies of the effect of age and sex upon basal heat production, the Haldane gravimetric method of determining the respiratory exchange of small animals (*16*) was used. The apparatus employed has been described in an earlier publication from this laboratory (*28*). For different-sized chickens different-sized chambers were employed, the endeavor being to fit the chamber snugly to the bird so as to minimize the possibility of considerable muscular activity and yet to permit the chicken to assume a comfortable posture.

In the earlier work, performed in 1923, no method of recording the activity of the birds had been elaborated, but in the later experiments (*29*) the animal chamber was suspended from a spring and the vertical movement of the chamber was recorded on a revolving "work-adder." The records thus obtained, which appear in Tables 4, 5, and 6 as "work record," are purely of comparative significance.

The heat production was computed by the well-known method of Zuntz and Schumburg (*25, p. 56–63; 39*), upon the assumption that the total respiratory quotient is nonprotein, thus neglecting the protein metabolism. From the available data relating to the calorific value of oxygen used in the combustion of the different food nutrients, it may be computed that the maximum error in the estimate of the total heat production resulting from this simplification is 6.58 per cent, while the usual errors would be less than 2 per cent.

EXPERIMENTAL DATA

The experimental results will be considered, not in the order in which they were obtained, but with reference to the age of the chicks. For this reason, some of the results to be considered later will be less satisfactory than some of those considered first, because of a better control of experimental conditions as familiarity with the methods increased.

The experiments with birds just after hatching were performed upon White Plymouth Rocks. The chicks were confined in small Mason jars and were under observation for three to seven hours, during which the respiratory quotient was determined. The CO_2 production during two or more 30-minute periods of quietness was found, and upon these values the basal heat production was computed. The surface area of the birds was estimated on the basis of 10 determinations of the skin area of as many White Plymouth Rock chicks 1 day old. The average weight of these chicks was 36 gm. and their average skin area, 91 sq. cm. In estimating the area of the experimental chicks, it was assumed that it would vary in proportion to the two-thirds power of the body weight. The average data from these tests are given in Table 1.

TABLE 1.—*The basal heat production of young White Plymouth Rock chicks*

Chick No.	Age	Condition	Body weight	Body surface	Temperature of chamber	O₂ consumed daily	Respiratory quotient	Heat production per day	
								Total	Per square meter body surface
	Days		Gm.	Sq. cm.	° C.	C. c.		Cals.	Cals.
1	1	Fasted since hatching	35.6	91	36.0	1,008	0.82	4.87	535
2	2	----do----	30.7	78	37.5	871	.86	4.24	544
3	3	----do----	32.9	83	37.5	959	.78	4.57	551
4	5	Fasted 36 hours	31.2	79	37.5	1,270	.73	5.97	756
5	6	Fasted 60 hours	31.3	79	37.5	934	.86	4.56	577
6	6	Fasted 65 hours	30.8	79	37.5	1,061	.90	5.22	661
7	13	Fasted 44 hours	25.3	64	37.5	899	.78	4.28	669
8	15	----do----	31.7	80	37.5	1,323	.83	6.39	799
9	16	Fasted 25 hours	30.8	78	37.5	1,354	.80	6.49	832
10	17	Fasted 43 hours	30.3	77	37.5	1,343	.75	6.35	825

Omitting the exceptional result for chick 4, the heat production per square meter of body surface increased regularly from the day-old chick to the 16-day-old chick. In an earlier publication (*28*) an average heat production of 703 calories per day per square meter of surface was observed with 28 mature Rhode Island Red hens, and one of 806 calories with 19 mature cocks. Evidently the chick immediately after hatching has a basal heat production considerably below the level of the mature chicken. These results are quite similar to those reported for infants.

In the next group of experiments concerned with the relation between age and basal heat production, young single-comb White Leghorn chicks fasted for 48 hours, were used. An attempt was made to reduce the activity of these birds to a minimum by wrapping them in cheesecloth, except for the head; the legs (in the squatting or roosting position) were bound somewhat loosely to the body. Two 3-hour periods were run upon each chick, one in the morning and one in the afternoon. In practically all cases, the production of heat was considerably higher in the morning than in the afternoon, the difference being the more marked with the younger chicks. In most cases where the rectal temperatures were observed, a distinct reduction of body temperature was noted from the time the chick was put in the chamber in the early morning to the time it was removed in the late afternoon. The exceptions were related generally to excessively high chamber temperatures in the afternoon, which were reflected in rectal temperatures of 108° to 110° F. It was at first thought that this reduction in metabolism was a diurnal phenomenon, and the procedure was therefore reversed with six chicks, the first experimental period being in the afternoon and the second in the morning following. In four of these experiments the morning heat production exceeded the afternoon, but in two cases the reverse was true.

The differences observed between morning and afternoon results (averaging 21 per cent with the youngest group and 12 per cent with the oldest) seemed too large to be a diurnal phenomenon, if one may judge from results on human subjects (*5*), and, moreover, they seemed to be in the wrong direction—with men the higher level appears to be in the afternoon. A more probable explanation is that

the difference was due to muscular activity. Quite possibly in the morning the chick struggled to some extent against its bandage, but in the afternoon had become either exhausted or reconciled to the experimental conditions. One experiment in which the bird was unwrapped in the morning but wrapped in the afternoon confirms this conclusion. During the morning the CO_2 excretion during a period of quietness (37 minutes) was measured, from which, in conjunction with the respiratory quotient for the three-hour period, it was computed that the heat production was at the rate of 10.38 calories per day. The value for the entire afternoon period, during which the bird was wrapped in cheesecloth, was 15.57 calories per day. This value expressed per square meter of body surface (1,188 calories) is clearly out of line with the other afternoon values for chicks of approximately the age of this one (13 days). It was therefore decided to discard the morning values in all of these experiments and to consider the lower afternoon values as more nearly approximating the basal heat production.

TABLE 2.—*The basal heat production of young single comb White Leghorn chicks*

Chick No.	Sex	Age	Body weight	Body surface	Body temperature	Maximum and minimum temperatures of chamber	O₂ consumed daily	Respiratory quotient	Heat production per day	
									Total	Per square meter body surface
		Days	*Gm.*	*Sq. cm.*	*°F.*	*°F.*	*Liters*		*Cals.*	*Cals.*
4776	Male	7	40.0	102		85–95	1.46	0.73	6.86	675
4540	do	8	43.4	107		85–95	1.85	.70	8.66	807
4761	do	9	38.7	99		85–95	1.23	.76	5.85	591
4733	Female	12	49.7	117		85–95	1.96	.75	9.28	790
4749	Male	13	58.2	130		85–95	2.19	.74	10.38	796
4953	do	14	60.8	134		85–95	1.46	.81	7.00	521
4891	do	16	62.4	137		85–95	1.85	.73	8.70	637
4505	do	19	86.0	169		85–90	3.92	.75	14.57	861
4762	do	20	76.8	157		85–95	3.64	.70	17.06	1,087
4947	do	21	82.9	164		85–95	4.07	.71	19.11	1,162
4604	do	22	71.8	150		87–92	3.19	.71	14.96	997
4849	do	23	94	179		85–95	4.03	.71	18.89	1,052
4670	do	27	126	218		80–90	4.82	.73	22.68	1,040
4628	do	29	147	242		80–85	7.34	.72	34.46	1,423
4387	do	30	128	220		80–88	4.70	.73	22.15	1,004
5000	do	98	730	932	106.4	92–94	13.66	.80	65.7	704
4717	do	104	820	1,008	106.4	89–92	14.28	.72	67.2	667
4900	do	106	1,010	1,180	106.4	92–95	19.38	.71	91.0	771
	Average									714
4511	Female	97	770	915	106.2	88–92	15.79	.73	74.4	813
4945	do	99	740	885	108.0	90–93	14.22	.73	67.1	758
4969	do	103	610	760	107.2	87–92	14.22	.73	67.0	881
4521	do	105	730	875	107.2	89–93	14.28	.76	67.9	776
4933	do	107	800	940	105.8	87–91	15.74	.72	67.0	713
	Average									788
4885	Male	146	1,500	1,580	104.8	72–80	25.41	.71	119.3	755
4832	do	149	800	995	105.0	70–78	14.50	.75	68.8	691
4564	do	152	1,390	1,530	105.0	70–76	27.05	.70	126.7	828
4755	do	154	1,600	1,620	104.8	76–84	22.96	.73	108.3	669
4865	do	161	1,390	1,530		78–84	19.54	.71	91.6	599
4900	do	163	1,650	1,640	106.0	76–82	26.66	.74	126.2	770
	Average									718
4858	Female	147	1,320	1,310	105.2	72–80	21.50	.71	100.8	769
4739	do	148	1,235	1,263	104.8	74–78	18.93	.71	88.8	703
4564	do	153	1,170	1,225	105.5	70–78	19.71	.70	92.4	754
4992	do	160	1,230	1,260	104.2	72–78	16.18	.71	76.0	603
4553	do	166	1,380	1,340	104.7	72–78	25.54	.70	119.7	890
	Average									744

TABLE 3.—*The average skin area of single comb White Leghorn chickens of different sex; each area is the average for 10 birds*

Body weight of cockerels	Average skin area of cockerels			Body weight of pullets	Average skin area of pullets		
	Body	Combs and wattles[a]	Total		Body	Combs and wattles[a]	Total
Gm.	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*	*Gm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*
[b]31. 1	86	----------	86	----------	----------	----------	----------
218	294	----------	294	222	325	----------	325
477	568	----------	568	468	602	----------	602
678	840	36	876	669	815	----------	815
874	986	66	1,052	890	1,016	----------	1,016
1,317	1,367	116	1,483	1,367	1,298	34	1,332
1,719	1,536	124	1,660	1,716	1,350	72	1,422
2,136	1,689	160	1,849	----------	----------	----------	----------

[a] The areas given are twice the areas outlined.
[b] The sex of these birds was not determined.

A summary of the data in this series of experiments is given in Table 2. In estimating the surface area of these chicks, advantage was taken of a considerable number of determinations of the skin area of single comb White Leghorn chickens made in the course of another investigation. In this investigation, the skin area of 10 chicks 1 day old was determined, and thereafter the skin areas of groups of 10 cockerels and 10 pullets at the approximate weights of 0.5, 1, 1.5, 2, 3, 4, and 5 pounds, except that no 5-pound pullets were measured. The areas refer to the body only, the skin of the shanks and feet not being determined. For the older birds, with combs and wattles of appreciable size, the area of these appendages was determined and multiplied by 2 to get their approximate surface area. The average skin areas of the different groups of chickens are given in Table 3. These areas were plotted upon coordinate paper against the body weights, and a separate curve was made for cockerels and pullets; the areas of the experimental birds was then estimated graphically from their body weights. It will be noted that with this group of birds, as with the group of young White Plymouth Rocks, the basal heat production per square meter of body surface starts at a low value and increases progressively. For these White Leghorns, the maximum basal metabolism is not reached for at least four weeks. In three months, the basal metabolic rate falls again to a level averaging 741 calories per square meter, which is maintained for at least five and one-half months.

The values for the later groups of birds do not reveal the expected sex difference, since the pullets showed on an average the greater basal metabolic rate. However, this is partly due to the fact that the area of the combs and wattles have been included in the surface areas used in these computations. Whether this is justifiable or not will depend upon the final explanation for the so-called "surface area" law; that is, whether it is based purely upon the mechanics of heat emission, or upon an anatomical relation between body surface and active protoplasmic material. If the areas of these appendages are not considered, the averages become 742 and 776 calories per square meter for the cockerels and 788 and 761 for the pullets.

TABLE 4.—*The basal heat production of White Plymouth Rock cockerels*

Date	Bird No.	Age	Weight	Rump to shoulder	Surface area	Maximum and minimum temperature of chamber	Work record	Oxygen consumed daily	Respiratory quotient	Heat produced daily		
										Total	Per kilogram body weight	Per square meter body surface
1923		*Days*	*Gm.*	*Cm.*	*Sq. cm.*	°*F.*		*Liters*		*Cals.*	*Cals.*	*Cals.*
Apr. 30	2082	35	278		320	73–87		9.4	0.81	45	164	1,419
May 1	2307	36	275		316	65–86		10.7	.71	51	184	1,601
May 3	2617	38	304		350	84		9.3	.89	46	151	1,314
May 4	2637	39	276		318	62–84		9.4	.80	46	165	1,431
Average		37	283		326			9.8	.80	47	166	1,441
June 6	2097	72	738	16.1	843	76–85		13.7	.77	65	88	770
June 7	2617	73	568	14.6	698	74–84		12.7	.70	60	105	860
June 9	2082	75	628	14.6	734	72–77		15.0	.70	70	112	960
June 11	2307	77	695	15.5	800	72–84		14.8	*a.*60	70	100	872
June 12	2637	78	640	14.3	732	74–86		11.6	*a.*68	55	85	744
June 13	2885	79	827	16.5	890	78–90		14.9	.70	70	85	789
Average		76	683		783			13.8	.69	65	96	832
July 24	2097	120	1,396	19.4	1,297	78–90		25.7	*a.*66	121	87	936
July 25	2885	121	1,443	19.4	1,319	74–83		26.9	.71	126	88	958
July 26	2307	122	1,074	18.3	1,099	76–84		18.0	.71	84	79	768
July 27	2308	123	1,504	18.8	1,321	78–83		24.2	.73	114	76	864
July 28	2009	124	1,201	18.5	1,169	75–82		19.8	.71	93	77	794
Average		122	1,324		1,241			23.0	.70	108	81	864
Sept. 24	2637	182	1,923	20.7	1,583	83–88		34.0	*a.*69	161	84	1,017
Sept. 25	2307	183	1,750	21.0	1,523	74–87		24.5	*a.*63	115	66	762
Sept. 26	2097	184	1,980	22.4	1,684	74–80		28.1	*a.*66	132	66	790
Sept. 27	2308	185	2,225	21.4	1,737	73–81		33.3	*a.*68	157	71	904
Sept 29	2009	187	1,761	20.4	1,502	68–81		26.2	*a.*69	123	70	821
Average		184	1,928		1,606			29.3	.67	138	71	859
Nov. 20	2308	239	3,025	22.1	2,065	68–86	13	42.6	.72	200	66	969
Nov. 21	2307	240	2,065	22.5	1,725	70–96	12	30.0	.72	141	68	817
Nov. 22	2097	241	2,792	23.6	2,063	71–90	8	37.2	.72	174	62	843
Nov. 23	2009	242	2,609	22.8	1,954	72–84	11	35.7	*a.*69	170	65	870
Nov. 24	2637	243	2,941	23.0	2,085	68–80	13	37.0	*a.*67	176	60	844
Dec. 1	2905	250	2,797	22.0	1,980	75–88	13	35.9	.71	166	59	838
Average		242	2,705		1,979			36.4	.70	171	63	864
1924												
Feb. 25	2862	336	2,322	20.3	1,719	80–94	17	36.3	*a.*69	171	74	995
Feb. 26	2097	337	2,490	23.3	1,934	54–90	6	29.7	.72	140	56	724
Feb. 27	2905	340	2,942	21.9	2,025	80–90	8	43.1	.73	203	69	1,002
Mar. 1	2009	341	2,764	23.4	2,043	78–92	5	43.1	.73	203	73	994
Mar. 2	2308	342	2,914	23.0	2,076	82–88	8	33.6	.71	158	54	761
Mar. 3	2637	343	2,934	23.0	2,083	78–86	3	29.4	.72	138	47	663
Average		340	2,728		1,980			35.9	.72	169	62	856

a In estimating the heat production for periods in which the observed respiratory quotient was less than 0.707, the latter respiratory quotient was assumed.

The last group of experiments to be considered on the relation between basal heat production and age and sex includes the first experiments undertaken on the gaseous exchange of chickens. In some respects the conditions under which these experiments were performed were not entirely satisfactory. However, they constitute the most complete set of experiments that has been carried out, and the results appear to be of great significance.

The chickens used were White Plymouth Rocks. At approximately monthly intervals these were removed from the range at the university poultry farm, brought to the laboratory in groups of five or six of like sex, and after a fast of 48 hours (except the first group of

cockerels, which were fasted for approximately 24 hours only), they were placed in a cylindrical animal chamber of appropriate size and subjected to a respiration test of 22 to 23 hours' duration. In most of the tests the activity of the birds during the period of observation was neither controlled nor observed, but the close quarters in which they were confined, combined with the complete darkness which prevailed within the animal chamber, appeared to have effectively discouraged any considerable muscular movements. The temperature of the laboratory, unfortunately, varied widely during these tests, as indicated by the records of a maximum and minimum thermometer placed within the animal chamber, and during the coldest weather it may be suspected that the heat production of the birds was raised to some extent above the basal.

TABLE 5.—*The basal heat production of White Plymouth Rock pullets*

Date	Bird No.	Age	Weight	Rump to shoulder	Surface area	Maximum and minimum temperature of chamber	Work record	Oxygen consumed daily	Respiration quotient	Heat produced daily		
										Total	Per kilogram body weight	Per square meter body surface
1923		*Days*	*Gm.*	*Cm.*	*Sq. cm.*	*° F.*		*Liters*		*Cals*	*Cals.*	*Cals.*
June 25	2458	91	668	15.2	775	81–86		11.1	0.76	53	79	684
June 26	2070	92	613	14.0	707	71–86		11.9	.74	56	92	800
June 27	2466	93	827	16.0	890	66–72		14.6	ᵃ.69	69	83	771
June 28	2441	94	779	16.9	892	67–72		16.3	.70	77	98	861
June 29	2581	95	684	15.3	788	70–76		12.6	.71	59	87	751
June 30	2191	96	821	16.3	896			15.5	ᵃ.68	73	89	816
July 2	2281	98	750		815	75–82		14.4	ᵃ.65	68	91	834
Average		94	735		824			13.8	.70	65	88	788
July 30	2441	126	1,069	17.4	1,063	78–84		16.7	.75	80	74	749
July 31	2070	127	839	16.1	809	80–87		11.9	.70	56	67	691
Aug. 1	2458	128	1,017	17.5	1,041	82–87		16.7	.70	78	77	753
Aug. 2	2466	129	1,022	16.5	1,007	80–86		16.3	.71	77	75	763
Aug. 3	2191	130	1,138	17.6	1,105	81–92		19.3	ᵃ.61	91	80	824
Aug. 4	2791	131	1,169	18.6	1,157	81–86		19.2	.71	90	78	782
Average		128	1,042		1,030			16.7	.70	79	75	760
Oct. 1	ᵇ 2191	189	1,597	19.8	1,404	70–76		27.0	ᵃ.68	127	80	907
Oct. 2	2458	190	1,392	18.9	1,275	69–74		21.9	.70	99	71	780
Oct. 3	2686	191	1,455	18.5	1,287	66–74		23.9	.71	112	77	871
Oct. 4	2070	192	1,006	15.9	977	64–73		13.9	.71	65	65	667
Oct. 5	2441	193	1,600	18.8	1,363	60–72		28.9	.70	135	85	994
Oct. 6	2791	194	1,651	20.0	1,437	57–66		28.9	.72	136	82	948
Average		192	1,450		1,290			24.1	.70	113	77	861
Nov. 28	2333	247	1,834	19.2	1,476	64–84	8	25.8	.70	121	66	819
Nov. 30	2441	249	2,177	18.8	1,590	48–82	6	29.9	.72	141	65	885
Dec. 2	2087	251	2,162	20.8	1,683	66–84	5	28.4	ᵃ.63	134	62	801
Dec. 3	2376	252	1,777	19.0	1,446	77–84	13	22.4	.61	106	60	737
Dec. 4	2686	253	1,716	19.0	1,420	74–82	3	20.6	.72	97	57	682
Dec. 5	2791	(254)	(2,066)	19.9	(1,602)	72–82	34	(34.1)	(.72)	(161)	(78)	(1,002)
Average		251	1,933		1,523			25.4	.68	120	62	785
1924												
Mar. 7	2866	347	1,677	19.3	1,417	80–88		25.7	.71	121	72	852
Mar. 13	2376	353	2,375	19.9	1,718	75–85	4	25.9	.71	121	51	707
Mar. 14	2333	354	1,906	18.1	1,454	74–86	3	28.3	.72	133	70	914
Do	2441	354	2,378	19.8	1,714		2	31.0	.72	146	61	852
Mar. 18	2694	358	2,265	20.5	1,708	82–94	4	26.7	.72	125	55	733
Mar. 23	2154	363	2,504	21.2	1,832	78–84	7	27.9	.78	131	52	716
Average		355	2,184		1,640			27.6	.73	129	60	796

ᵃ In estimating the heat production for periods in which the observed respiration quotient was less than 0.707, the latter respiration quotient was assumed.

ᵇ This bird was fasted for 29 hours only.

If the birds originally selected for this work died or were used for other purposes, they were replaced by others of the same breed and sex. The experimental observations summarized in Tables 4, 5, and 6 refer to groups of cockerels, pullets, and capons.

TABLE 6.—*The basal heat production of White Plymouth Rock capons*

Date	Bird No.	Age	Weight	Rump to shoulder	Surface area	Maximum and minimum temperature of chamber	Work record	Oxygen consumed daily	Respiratory quotient	Heat produced daily		
										Total	Per kilogram body weight	Per square meter body surface
1923		*Days*	*Gm.*	*Cm.*	*Sq. cm.*	*° F.*		*Liters*		*Cals.*	*Cals.*	*Cals.*
July 3	2085	99	870	16.0	912	77–81		15.3	^{a}0.61	72	83	788
July 6	2207	102	916	16.4	950	83–91		16.4	^{a}.64	77	84	814
July 7	2870	103	1,020	17.2	1,032	82–90		19.2	.74	91	90	878
July 8	2365	104	936	17.3	992	80–90		16.2	.78	77	83	780
Average		102	935		971			16.8	.69	79	85	815
Aug. 6	2207	133	1,258	17.2	1,146	80–94		18.0	.73	85	67	739
Aug. 7	2870	134	1,324	18.6	1,232	79–87		20.5	^{a}.67	96	73	782
Aug. 8	2085	135	1,239	17.7	1,157	78–93		20.0	.70	94	76	812
Aug. 9	2365	136	1,229	18.7	1,190	82–93		16.5	^{a}.67	78	63	652
Aug. 10	2364	137	1,847	19.9	1,515	85–94		31.3	.72	147	80	970
Aug. 11	2022	138	1,152	19.6	1,186	85–92		21.4	.72	100	87	847
Average		135	1,341		1,238			21.3	.70	100	74	800
Oct. 9	2022	197	1,296	20.7	1,299	70–80		22.8	.73	108	83	828
Oct. 10	2365	198	1,934	19.9	1,550	78–90		16.5	.74	78	40	501
Oct. 11	2085	199	1,306	20.5	1,297	73–80		20.1	.71	95	72	730
Oct. 12	2364	200	2,517	22.5	1,904	71–78		37.7	.72	178	71	933
Average		199	1,763		1,512			24.3	.72	114	66	748
Dec. 10	2364	259	3,297	23.2	2,219	62–88	3	46.1	.72	213	65	960
Dec. 11	2365	260	2,234	20.1	1,676	62–88	3	19.7	.72	92	41	551
Dec. 12	2022	261	1,629	21.1	1,474	74–78		23.2	^{a}.62	109	67	739
Dec. 14	2695	263	2,493	23.0	1,920	68–82	13	29.7	.71	139	56	724
Dec. 15	2085	264	1,509	19.8	1,365	72–94	7	19.7	.70	92	61	677
Dec. 19	2132	268	2,437	23.3	1,913	80–84	24	31.6	.71	148	61	772
Average		262	2,266		1,761			28.3	.70	133	59	737
1924												
Mar. 24	2733	364	2,610	21.7	1,897	72–86	1	30.3	.70	142	54	748
Mar. 25	2132	365	3,105	22.7	2,126	68–80	9	29.7	^{a}.68	140	45	658
Mar. 26	2272	(366)	(2,277)		(1,705)	78–98	17	(33.0)	(.74)	(156)	(68)	(915)
Mar. 27	2725	367	2,955	23.2	2,101	78–85	7	37.7	.71	176	60	840
Mar. 28	2364	368	3,864	23.8	2,440	81–86	4	45.4	.73	213	55	874
Mar. 29	2412	369	3,379	21.3	2,135	79–84	5	34.2	.73	161	48	756
Average		366	3,183		2,140			35.5	.71	166	52	775

^{a} In estimating the heat production for periods in which the observed respiratory quotient was less than 0.707, the latter respiratory quotient was assumed.

The last-named were caponized at 70 days of age. The surface areas of these birds, except those of the youngest group, were estimated from their body weights and their measurements from the shoulder to the rump, according to the formula (*30*) $S = 5.86\ W^{0.5} L^{0.6}$, in which S is the surface area in square centimeters, W the body weight in grams, and L the rump-to-shoulder distance in centimeters. The areas of the youngest group of cockerels were estimated from the determined areas of five 0.5-pound birds, on the assumption that the area varied as the two-thirds power of the body weight. For convenience in studying the large numbers of data in these tables, the group averages have been summarized in Table 7.

TABLE 7.—*A summary of the average results in Tables 4, 5, and 6*

				Heat produced daily		
Num ber of birds	Age	Weight	Surface area	Total	Per kilo- gram body weight	Per square meter body surface
	Days	*Gm.*	*Sq. cm*	*Cals.*	*Cals.*	*Cals.*
COCKERELS						
4	37	283	323	47	166	1,441
6	76	683	783	65	96	832
5	122	1,324	1,241	108	81	864
5	184	1,928	1,606	138	71	859
6	242	2,705	1,979	171	63	864
6	340	2,728	1,980	169	62	856
PULLETS						
7	94	735	824	65	88	788
6	128	1,042	1,030	79	75	760
6	192	1,450	1,290	113	77	861
5	251	1,933	1,523	120	62	785
6	355	2,184	1,640	129	60	796
CAPONS						
4	102	935	971	79	85	815
6	135	1,341	1,238	100	74	800
4	199	1,763	1,512	114	66	748
6	262	2,266	1,761	133	59	737
5	366	3,183	2,140	166	52	775

The first group of cockerels, averaging 37 days of age, showed a relatively intense basal heat production. Apparently these birds were near, if not at, the peak of rising basal metabolism (per unit of surface) noted in the preceding studies on young chicks. Between the age of this group and the age of the next group (76 days) the intensity of the basal metabolism apparently suffered a rapid decline. In this connection some results on a group of six Rhode Island Red chicks, averaging 55 days of age, may be considered. (See Table 8.)

TABLE 8.—*The basal heat production of a group of young Rhode Island Red chicks*

Date	Bird No.	Age	Weight	Sur- face area	Maxi- mum and mini- mum temper- ature of cham- ber	Oxy- gen con- sumed daily	Respi- ratory quo- tient	Heat produced daily		
								Total	Per kilo- gram body weight	Per square meter body surface
1923		*Days*	*Gm.*	*Sq. cm.*	*° F.*	*Liters*		*Cals.*	*Cals.*	*Cals.*
June 18	C429	53	240	345	81–88	6.48	0.70	30.4	126	881
June 19	C385	54	238	340	80–89	6.61	.69	31.0	130	912
June 20	C485	55	223	325	82–91	6.68	a .70	31.3	140	9'3
June 21	C383	56	200	300	82–92	5.90	.70	27.6	137	920
June 22	C491	57	283	390	83–93	7.64	a .70	35.8	127	918
June 23	C___	58	250	360	_____	7.16	.69	33.4	134	928
Average		55	239	343	_____	6.74	.70	31.6	132	920

a Assumed. The observed respiratory quotient was considerably smaller.

The experiments on these chicks were conducted at about the same time as the other experiments, and exactly the same procedure was followed.   The sex of these birds was not recorded.   The average basal heat production for the group was 920 calories per square meter of body surface (estimated from skin area determinations of White Plymouth Rock birds of approximately the same size), the individual determinations being remarkably concordant.   These results, in comparison with those obtained on the two youngest groups of White Plymouth Rock cockerels, indicate that the intensity of metabolism which had reached its peak at 30 to 40 days of age was greatly reduced at 55 days, though still distinctly higher than the level observed three weeks later.

It is a matter of considerable significance that, for the cockerels and pullets, the basal heat production per square meter of body surface was remarkably constant for all age groups, except the youngest cockerels, while the basal heat production per kilogram of body weight decreased continuously with advancing age.   This fact constitutes a formidable argument in favor of the body surface as a unit of reference for basal metabolism as opposed to the body weight.

Whatever the ultimate explanation of the relation between basal heat output and body surface may prove to be, it is undoubtedly the closest relation involving a factor of body size that has thus far been discovered.   It has been pointed out previously (13) that it is contrary to fact to assume, as is frequently done in explaining the surface area law, that the active protoplasmic tissue varies in proportion to the body surface.[4]   On the other hand, it appears reasonable to suppose that in the evolution of species the intensity of the basal heat production should be adjusted to such a level that, at temperatures so extreme as to threaten the continued life of the animal, the physiological mechanism for restricting heat emission at low temperatures or increasing it at high temperatures should be equally effective among animals differing in size. This would be true if the basal heat production per unit of surface were approximately the same for all (warm blooded) animals, since the extent of surface is the main anatomical factor determining heat emission, either by radiation, the method of heat loss prevailing at low temperatures, or by the evaporation of water, the only method operative at environmental temperatures above that of the animal body itself.

In previous experiments (28) with mature Rhode Island Red cocks and hens, average values of 806 and 703 calories were obtained. The same method was used and much the same conditions prevailed as in the work with the White Plymouth Rocks.   Whether these lower values, especially for female birds, are due to the greater age of the birds, to a lesser activity, or to a more prolonged confinement in the laboratory can not at present be determined.   In the hope of throwing some light upon this point, basal metabolism studies were made with four mature White Plymouth Rock hens.   The periods of observation were short (30 to 50 minutes) and were taken when the hen was completely at rest.   Only the CO_2 production was deter-

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[4] The same situation exists in comparing man with the chicken as in comparing man with the rat. Thus, a man of average height (173 cm.) and weight (70 kgm.) possesses a surface area (1.83 sq. m.) nine and two-tenths times as great as the surface area of a White Leghorn cockerel weighing 2 kgm. If the protoplasmic matter in animals varies with the body surface, the man should contain only 18 kgm., or 26 per cent, of protoplasm, on the extreme assumption that the cockerel consists entirely of active cellular tissue

mined; in the computation of the heat production, a basal respira-
tory quotient of 0.73 was assumed. Several determinations were
made upon each hen. The results are summarized in Table 9.

TABLE 9.—*The resting metabolism of mature White Plymouth Rock hens*

Hen No	Body weight	Esti- mated body surface [a]	Temper- ature of chamber	CO_2 per hour					Heat produced daily	
				1	2	3	Average	Total	Per square meter body surface
	Grams	*Sq. cm.*	*°F.*	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*	*Calories*
35	2, 290	1, 757	78–86	1. 52	1. 42	--------	1. 47	116	661
488	2, 900	1, 961	68–82	1. 48	1. 31	1. 37	1. 39	109	558
50	2, 870	2, 006	78–84	1. 51	1. 62	1. 53	1. 55	123	611
48	3, 000	2, 011	82–84	1. 84	1. 78	1. 60	1. 74	137	684

[a] The surface area was estimated from the body weight and the rump-to-shoulder dimension.

Quite possibly the difference between the heat production per
square meter of surface for these hens (averaging 628 calories)
and the average obtained for the mature Rhode Island Red hens
(703 calories), if significant, is due to a less degree of activity. In
any case, it appears probable that the values obtained for the young
White Plymouth Rocks (852 calories for cockerels and 805 calories
for pullets) are subject to a considerable decrease at a later age.

Comparing the average basal metabolism per unit of surface for
the different groups of cockerels with those for the groups of pullets
of approximately the same age, it appears that in four of the five
possible comparisons the average for the cockerels exceeds that for
the pullets. The opposite relation for the groups with average
ages of 184 and 192 days may quite probably be due to the low
temperatures prevailing during part of the day throughout the
observations on the pullets. (See Table 5.) This may have raised
the heat production of the pullets of that group above the actual
basal level. The average value for all groups of cockerels but the
first was 852 ± 12 calories per day per square meter of surface, and
that for the pullets was 805 ± 10 calories. The average difference
is 47 ± 16 calories. The basal metabolism of the pullets thus aver-
aged 5.5 per cent less per unit of surface than the basal metabolism
of the cockerels.

The coefficient of variation of the 28 determinations of the basal
metabolism per unit of surface for the cockerels was 11.3 per cent,
and for the pullets 10.1 per cent. These coefficients may be compared
with those reported by Harris and Benedict (*19*) for men and women,
i. e., 8.05 and 9.17, respectively; with those reported by Mitchell and
Carman for rats (*27*), i. e., 13.5 for males and 8.0 for females; and
with those observed by Mitchell and Haines (*28*) for mature Rhode
Island Red chickens, i. e., 10.9 for cocks and 11.6 for hens. Thus,
although the experimental conditions in the work on the White
Plymouth Rock chickens were not under as good control as would
be desirable, still the varying conditions of temperature and activity
apparently did not induce an abnormally great variability in the
results secured. It is fair to conclude, therefore, that the average

basal heat productions obtained were not seriously vitiated by such uncontrolled factors.

The calorimetric results obtained upon the capons have a bearing upon the question of the effect of castration upon metabolism. Although many reported experiments (*1, 22, 23, 24*), particularly those of Heymans (*22*) on cocks, afford evidence in favor of the view that male castration lowers the basal metabolism, other experimental observations are on record (*2, 12, 26*) in which no evidence of such an effect appears. The writers' results speak unmistakably in favor of the view that removal of the testes lowers the basal metabolism. From Table 7, it is evident that the group averages for the capons decreased progressively with increasing time after castration (which occurred at 70 days of age), except for the last group. For seven of the capons, more than one observation was made. These have been summarized for each bird separately in Table 10.

TABLE 10.—*Effect of castration on basal metabolism*

Capon No.	Age	Basal metabolism per square meter body surface	Capon No.	Age	Basal metabolism per square meter body surface
	Days	*Calories*		*Days*	*Calories*
2085	99 135 199 264	788 812 730 677	2365	104 136 198 260	780 652 501 551
2207	102 133	814 739	2364	137 200 259 368	970 933 960 874
2870	103 134	878 782	2022	138 197 262	847 828 739
2132	268 365	772 658			

It is evident that for each capon, with the exception of No. 2364, a marked lowering of metabolism occurred, the decrease extending over several months. No such decrease in basal metabolism was noted with the cockerels or the pullets. Against the possible explanation that the decrease was due to a lowering of muscular activity, it may be pointed out that in the later experiments in which activity records were secured, the indicated activity of the capons (Table 6) was certainly no less than the indicated activity of the cockerels (Table 4) or of the pullets (Table 5). The lowered metabolism seems to be a true effect of castration.

The average basal metabolism of the capons was 779 ± 15 calories per day per square meter of surface, and the average difference between cockerels and capons in this value was 73 ± 19. This appears to be a significant difference, statistically. Actually, the difference between cockerels and capons was greater than this, since the lowering of metabolism induced by castration was not immediately initiated, and since it extended over several months. The maximum effect was probably measured in the group of capons averaging 262 days of age. The average value in this group was 737 calories, as compared with the average of 852 calories for the cockerels, a reduction of 13.5 per cent.

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SUMMARY

The effect of age on the basal heat production of chickens has been studied, using several different breeds of birds. A remarkable similarity was observed between chickens and human subjects, in that the metabolism per unit of surface is distinctly below the adult level at hatching (birth), rises rapidly to a maximum, and then decreases again to an adult level that is maintained for a considerable fraction of the life span. For the chicken, the peak in the curve appears to be reached at 30 to 40 days of age, and the adult level at about 70 to 80 days.

The basal metabolism of chickens per unit of weight decreases continuously from the peak age to 1 year of age at least. For this species of animals the greater constancy of the basal heat production, when referred to surface than when referred to weight, is distinct and marked.

The basal metabolism of cockerels is eventually higher than that of pullets, though the exact age at which the sex difference appears has not been determined. In a group of White Plymouth Rock chickens ranging in age from 72 to 363 days, the average basal metabolism of the cockerels was 852 ± 12 calories per day per square meter of surface, and that of the pullets was 805 ± 10 calories. At more advanced ages there appeared to be a further reduction in basal heat production of 6 to 12 per cent for cocks and hens, respectively.

The castration of cockerels lowers their basal metabolism, though the effect may not be immediate and, when initiated, may extend over several months. The average maximum effect observed was a reduction of 13.5 per cent.

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THE INHERITANCE OF OVATE AND RELATED SHAPES OF TOMATO FRUITS[1]

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INTRODUCTION

Ovate or elliptical shapes of tomato fruits, characteristic of such commercial varieties as the Red or Yellow Plum and similar types, including the Red or Yellow Pear varieties, occupy an intermediate position in the matter of inheritance. They are not inherited as distinctly as are the color characters of the fruit or certain morphological characters (dwarf, peach, leaf types, inflorescence types), nor are they as complex genetically or as susceptible to environmental agencies as the more quantitative character of fruit size. From a genetic standpoint, they may be termed semiquantitative in nature.

Circumstantial evidence, verging on complete proof, has indicated that the inheritance of ovate shape in tomato fruits may conveniently be explained as being controlled largely by a single, major gene carried in the first linkage group of Lycopersicum (*3*).[3] Experimental data are now available to confirm this, and to afford the necessary quantitative demonstration of the situation.

METHODS

Perhaps the most direct, accurate, and concise description of shape in tomato fruits is given by the ratio of the measured equatorial diameter of the fruit (taken midway between the stem and blossom end) to the polar diameter, taken at right angles to the equatorial. This ratio may be expressed as an index number. Accordingly, in this report the term "shape index" is defined as the following ratio:

$$\text{Shape index} = \frac{\text{equatorial diameter}}{\text{polar diameter}}$$

With such an index, a round fruit will be rated at approximately 1.00, an ovate type about 0.75, and an oblate fruit will vary from 1.15 upwards. Shape measurements were made with small steel calipers, readings being taken to the nearest millimeter.

Up to the present the writer has not attempted to correct the polar diameter measurements in fruits possessing a deep cavity or basin at the stem end. With such fruits care was taken to place the calipers in such a manner as to give an average reading for the ofttimes irregular surface of the fruit. Accordingly, the shape indices of the larger oblate fruits with deep cavities will be smaller than if the

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 10
May 15, 1927
Key No. Ia.–13

(961)

measurement had been taken at the stem scar. This minimization
of the shape index of the larger fruits is not considered as seriously
affecting the general shape relationships. It merely affects the
frequency distribution or curve at the upper end a trifle, and the
results are probably a bit conservative if differences are being con-
sidered as they are in this report.

Ordinarily eight or more tomato fruits from a single plant are
measured. In some earlier work only five fruits were used, but this
number has now been increased so that 10 are used if possible, since
this simplifies the calculation of averages. The tomato plants are
trained to a single stem in the greenhouse, and the fruits to be meas-
ured come from the first four or five clusters. In the field very little
pruning is practiced until the first five clusters have set, after which
the tops are pruned rather severely.

In the field the plants of a series are replicated in short rows, 10
plants to the row. In the greenhouse a lot of plants (usually
200 to 400) are grown in 8-inch pots, and are shifted five or six times
to various places on the greenhouse benches so as to equalize growing
conditions as much as possible.

<center>EXPERIMENTAL DATA</center>

The inheritance of the ovate fruit shape will be considered first,
followed by the genetic relations of this shape factor to other factors,
such as dwarf, peach, and fruit size. In a later section the mode of
inheritance of the oblate and round fruit shapes will be presented,
primarily for comparison with the behavior of ovate shape.

<center>INHERITANCE OF OVATE FRUIT SHAPE</center>

The commercial varieties (with the abbreviations) involved in the
study of ovate fruit shape were as follows:

Oblate types	Ovate types	Round type
New Globe (NG).	Yellow Plum (YP).	Red Peach (RP).
Dwarf Stone (DS).	Red Pear (RPr).	

These varieties have been crossed under controlled conditions, and
F_1, F_2, and back-cross generations have supplied the material for the
measurements.

The elliptical or ovate shape was introduced into the hybridization
tests by the Yellow Plum variety. Two oblate and one round or
roundish-oblate variety served to give the contrasting character in
the three crosses that are listed in Table 1, which gives the shape
indices of the P_1, F_1, and F_2 generations. In all cases each of these
three generations has been grown and compared during the same
season and under similar conditions.

TABLE 1.—*Mean fruit shape indices of three crosses of ovate by oblate varieties*

Crosses	Ovate parent	Oblate parent	F_1	F_2	Where and when grown
Yellow Plum×New Globe	0.71	1.22	1.08	1.06	Field, 1926.
Yellow Plum×Dwarf Stone	.75	1.34	1.15	1.13	Greenhouse, 1920
Yellow Plum×Red Peach	.71	1.15	.98	.97	Field, 1926.

⅃ As measured by the shape indices, the first hybrid generation shows an intermediate condition in all the crosses. The fruits tend to be more or less round. In all cases, however, there is a slight dominance of the oblate shape. The F_2 generations exhibit an average condition approaching the F_1 shape, but naturally this generation is highly variable, as may be noted in the subsequent graphs (fig. 1). Both parental shapes are usually recovered in this F_2 generation, a situation which ordinarily does not occur when fruit sizes are involved in tomato hybrids. The F_2 data of the three frequency distributions are plotted in Figure 1, the solid line in the graphs representing the total F_2 frequency.

Whereas all three curves in this figure approach a normal curve, there is sufficient evidence of multimodality to occasion some consideration of these F_2 distributions. To one with a predilection for a simple, monohybrid explanation of the case, there is a hint of trimodality in these curves, suggestive of a $1:2:1$ distribution. The secondary modes on both sides of the major modes may be explained away as modifying factors of some sort. To the skeptic, however, the curves probably exemplify the capricious fluctuations of such characters as shape. At any rate there would be serious difficulty in attempting to define the limits of the three genotypes in a monohybrid population. Apparently F_2 distributions of shape characters are uncertain measures of hereditary genotypes, whether caused by environmental influences or germinal complexity. Accordingly, one must turn to other devices to interpret the case.

The solution of the difficulties noted above proceeded from a consideration of the back cross of the F_1 of an ovate-oblate cross to the ovate parent. Several F_1 plants of the Yellow Plum-Dwarf Stone cross were reciprocally crossed by the Yellow Plum type. They were also crossed with the unrelated, ovate (pyriform) type, the Red Pear.

Shown in Figure 2 are the curves of the two back crosses. In the Yellow Plum back-cross generation of 276 plants there is exhibited a bimodal curve of unusual distinctness. The conclusion is evident that two major factors are acting with pronounced effect, there being no evidence of secondary or minor influences beyond the fluctuations to be expected in such characters as shape. Presumably two hereditary genotypes are responsible for the two modes at the classes 0.80 and 1.10. This pointed suggestion, coupled with the hint of trimodality in the F_2 generations, leads to the reasonable deduction that ovate shape in tomato fruits, when allelomorphic to the oblate shape, may very well be governed by a single, major gene. The back-cross condition of bimodality would then represent the action of the heterozygous and the homozygous recessive genotypes.

Verification for such a deduction is afforded by the cross of similar F_1 plants (Yellow Plum × Dwarf Stone) to the ovate (and pyriform) Red Pear variety. Such a cross involving 89 plants is shown graphically in the upper part of Figure 2. Again an equally distinct, bimodal curve results, giving evidence that the pyriform shape of the Red Pear variety is genetically equivalent to the ovate shape of the Yellow Plum as far as the ellipsoidal features of its shape are concerned.

It is probable that pyriform and true ovate shape differ only in a secondary manner. Ovate shape seems to be incompletely domi-
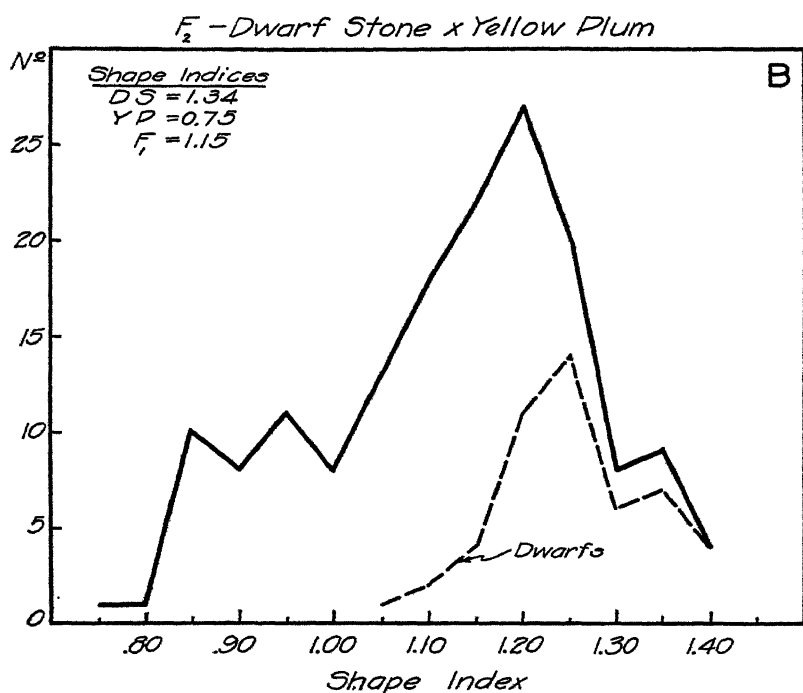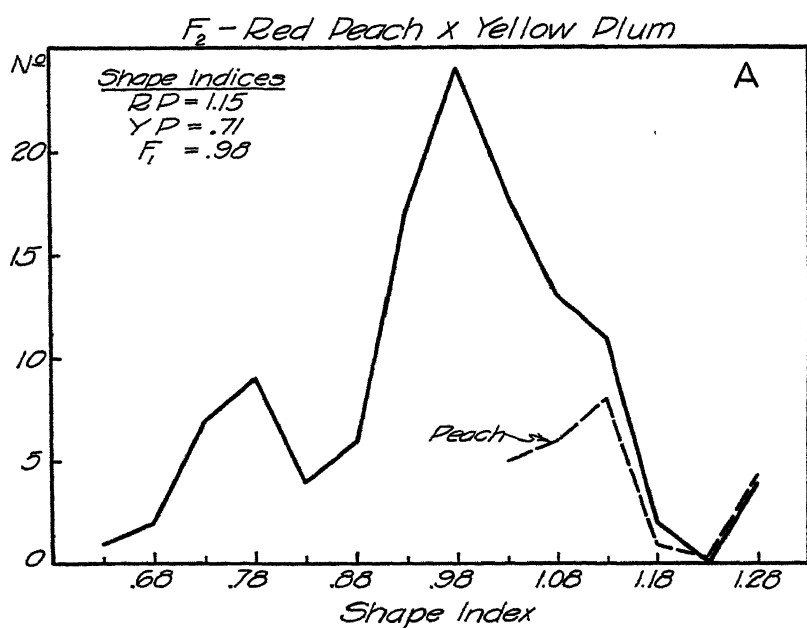
Fig. 1.—Frequency distributions of three different F_2 generations from crosses of ovate×oblate varieties, showing tendency for trimodaity in fruit shape; solid line represents total F_2 frequency

nant over pyriform shape. Very often in crosses of oblate × ovate (nonpyriform), a few pear-shaped types appear, despite the fact that both parents were homozygous for their respective fruit shapes. In the New Globe × Yellow Plum cross discussed herein, for example, the following proportion of pear fruits was observed: 99 oblate or round; 24 ovate (nonconstricted); 12 pyriform (0 distinct, 5 medium, 7 slight pear-shaped). This seems to indicate that oblate varieties may sometimes carry pyriform shape as a cryptomere, since there is other evidence to prove that the homozygous Plum varieties are not responsible for the pear shape.

This suggestion is confirmed in the back cross of the F_1 of the Dwarf Stone-Yellow Plum cross to the Red Pear variety, in which the following distribution of shapes occurred: 41 oblate and round; 24 ovate (nonpyriform); 9 slight pyriform; 13 medium pyriform; 2 distinct pyriform. Apparently one half of the elliptical fruits showed

F_2 – New Globe × Yellow Plum
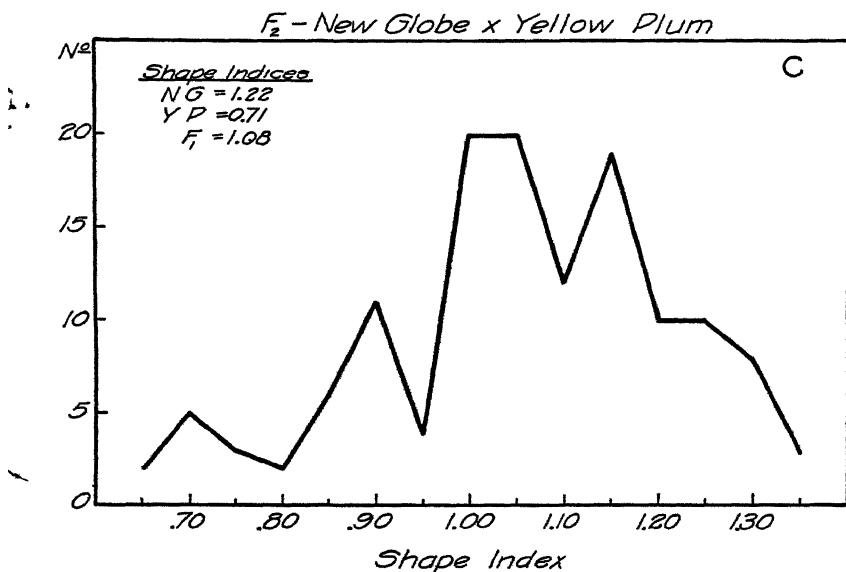
FIGURE 1—Continued

some constriction of the neck (pyriform), the other half not. This suggests that the ovate gametes from the F_1 of an oblate × ovate cross (such as Dwarf Stone × Yellow Plum) are of two sorts, one with the true ovate gene coming from the P_1 Yellow Plum variety, and the other with a pyriform gene which probably was contributed by the oblate Dwarf Stone variety.

It is also worth noting that in some crosses of oblate by pyriform types the pear shape certainly does not appear in F_2 in its full quota of 25 per cent, as might be expected from the results of MacArthur (5). There is always a marked deficiency of true pear-shaped fruits in F_2. For example, in two crosses for which only descriptions of the fruit shapes are available the proportion of pyriform types was as follows:

F_2 — Ponderosa × Red Pear — 28 nonpyriform; 3 pyriform.
F_2 — Dwarf Peach × Red Pear — 190 nonpyriform; 20 pyriform (including 4 distinct, 7 medium, and 9 slightly constricted types).
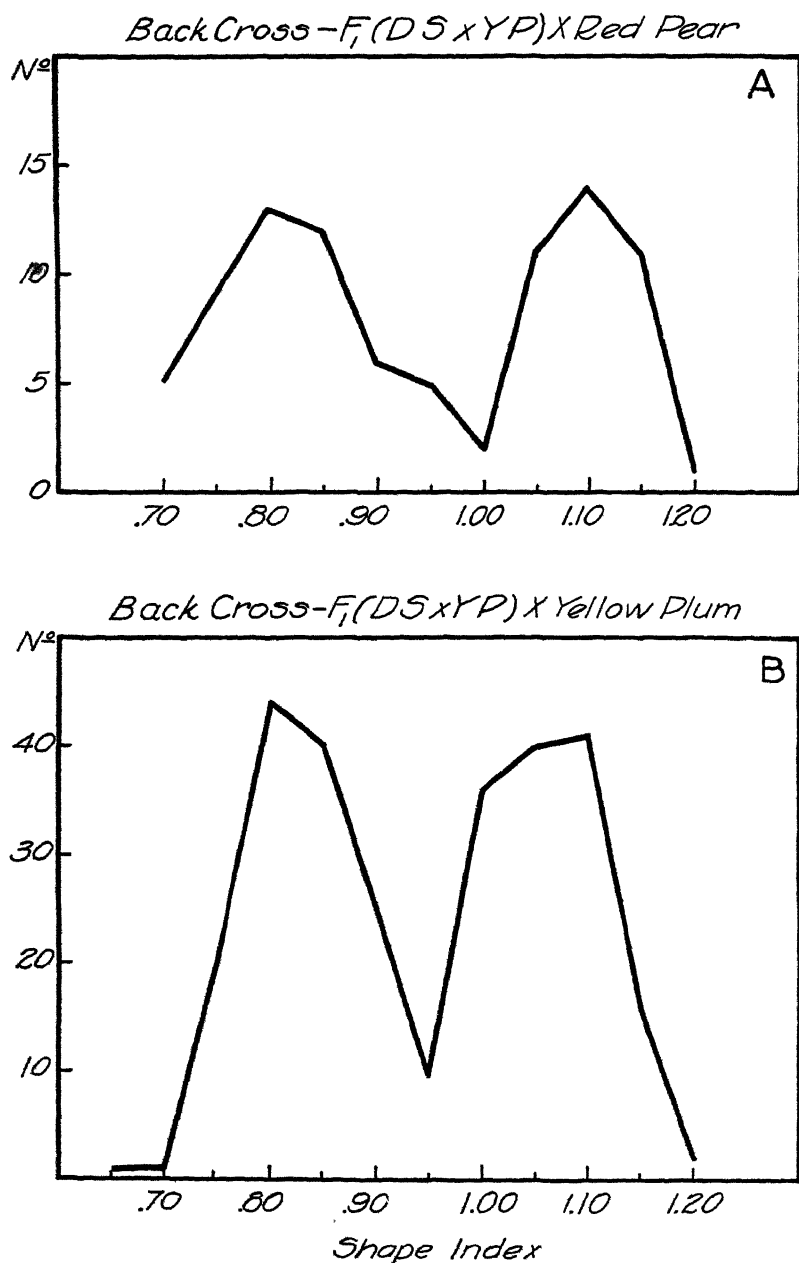
FIG. 2.—Two back-cross generations showing distinct bimodality in fruit shape. F_1 plants of the Dwarf Stone×Yellow Plum cross were crossed to the ovate Yellow Plum (below) and also to the pyriform Red Pear (above). The plants were grown in the greenhouse at the same time as the F_2 generation in Figure 1

Among the nonpyriform fruits in these F_2 generations are classed the true ovate or plum types. Were these added to the pyriform types, the combined percentage would approximate the expected 25 per cent. These results confirm the previous findings that ovate and pear shapes are conditioned by the same hereditary factor as far as their their ovate shape is concerned; in which case it is presumably not the pyriform gene but the ovate gene that is the simple allelomorph of the oblate shape gene. The factor for pyriform shape accordingly belongs elsewhere, and as far as the writer is aware, its true genetic status is practically unknown. It is possible that MacArthur's (5) report on the simple recessive nature of the pear gene differs from the writer's conception of the case by reason of the use of the Yellow Pear variety by MacArthur as the source of his pyriform gene. It is difficult to conceive, however, that the Yellow Pear variety, which is identical in appearance with the Red Pear variety used herein, should carry a different sort of shape gene. The situation needs further work. As far as the writer is concerned, however, the main point has been established, namely, that ovate and pyriform shapes act similarly from a genetic standpoint in crosses with the oblate types, indicating that they possess the same ovate-determining gene.

If this is true it is more than likely that ovate shape in tomato fruits is in general governed by a single Mendelian factor, at least when studied in contrast with the oblate or roundish-oblate fruit shapes. Despite its semiquantitative nature, it can be isolated in back crosses at least, sufficiently delimited to afford some reasonable assurance of its presence. Under such circumstances its exact location in a particular linkage group becomes possible.

The genetic symbol for this ovate gene might be given as *oo*, since this report is the first to demonstrate its existence quantitatively. This symbol was suggested in an earlier paper (3), but an abstract by MacArthur (4) had suggested the symbol *pr* for the pyriform shape, inferring that this was the gene located in the first linkage group. Since this shape with the constricted neck is now known to be genetically similar to the ovate form, it is probable that the latter is the true shape gene residing in the first chromosome. As the matter now stands the use of the *Oo* symbols as factors for the oblate-ovate relation is the more descriptive and fundamental, and also the use of a biliteral symbol *pr* is likely to prove unwieldly in view of a more complex situation in fruit shape about to be described. In this case, multiple allelomorphs are probably concerned which will necessitate at least a triliteral symbol for each of the fruit shapes concerned. Accordingly, it is more advantageous to use the simplest symbolism, waiving for this reason the doubtful correctness of the *pr* factor symbol.

<center>LINKAGE OF OVATE FRUIT SHAPE AND TALL-DWARF FACTORS</center>

Since it has been demonstrated by a quantitative analysis that ovate fruit shape is probably governed by a single, major gene, it is possible to ascertain more or less exactly the linkage relations of this gene by means of the same quantitative measurements of fruit shape. Earlier work by Lindstrom (3) and by MacArthur (5) has indicated that the ovate gene belongs in the first linkage group of

the tomato. Accordingly it should exhibit linkage with the tall-dwarf (*Dd*) and smooth-peach (*Pp*) factor pairs.

Experimental data on the linkage between the tall-dwarf and ovate genes are now available from the cross of the Yellow Plum by Dwarf Stone varieties. Both F_2 and back-cross results have been procured in this cross. The back-cross data, being more reliable will be presented first.

TABLE 2.—*Linkage relations between fruit shape and dwarf factors in reciprocal back crosses of the Dwarf Stone × Yellow Plum F_1 by Yellow Plum tested by seedling progenies*

Shape index	F_1 × Yellow Plum				Yellow Plum × F_1				Grand total
	DD	Dd	Untested	Total	DD	Dd	Untested	Total	
0.68					1			1	1
0.71					0			0	0
0.74	1			1	2			2	3
0.77	5	1		6	5		1	6	12
0.80	3	1		4	14	2		16	20
0.83	12	2		14	16	1		17	31
0.86	14	3		17	8	3		11	28
0.89	11	0		11	7	1		8	19
0.92	2	0		2	9	2	1	12	14
0.95	4	0		4	2	0		2	6
0.98	1	1		2	3	2		5	7
1.01	2	5		7	2	6		8	15
1.04	3	10	1	14	1	13		14	28
1.07	1	12	1	14	2	11	1	14	28
1.10	2	8	0	10	0	19		19	29
1.13	1	3	1	5	0	13		13	18
1.16	2	7		9	1	3		4	13
1.19		1		1		1		1	2
1.22						2		2	2
Total	64	54	3	121	73	79	3	155	276

In Table 2 are given reciprocal back-crosses of F_1 (DS × YP) × Yellow Plum. Since no double-recessive (ovate-dwarf) type was available at the time this material was grown, it became necessary to use the Yellow Plum (ovate-tall) variety. This requires a further seedling test to determine the segregation of the *Dd* genes. This is easily done, since tall and dwarf types may be determined in the seedling stage without difficulty or uncertainty. In Table 2, accordingly, 270 progenies of seedlings were grown from the back crosses noted above. At least 30 seedlings were used for each progeny; usually the average was 40 or more. In cases of doubt, arising ordinarily because of small or abnormal seedlings which might be confused with dwarfs, two or three plantings of 40 seedlings each were grown. With 40 seedlings as a test for *DD* or *Dd* genotype on the basis of a 3 : 1 ratio, the probability of mistaking the genotype by the chance nonappearance of a dwarf, is only 0.05 per cent.

Because of the bimodal nature of the shape curve (see fig. 2) it was relatively simple to draw a reasonably sharp line between ovate and nonovate fruit shapes. From an inspection of Table 2, one may note that very few of the ovate fruit types were of the *Dd* genotype, and but few of the nonovate types of the *DD* genotype, these representing the nonparental or crossover combinations. These data have been summarized in Table 4, where one may note that of the 270 back-cross individuals tested in these reciprocal crosses, only 33 were of the nonparental type. This gives a total crossing-over

percentage of 12.2 (or 9.9 per cent crossing over in microsporogenesis and 15.2 per cent in megasporogenesis).

TABLE 3.—*Linkage relations between fruit shape and dwarf factors in back cross of F_1 (Yellow Plum×Dwarf Stone) by Red Pear tested by seedling progenies*

Shape index	DD	Dd	Total	Shape index	DD	Dd	Total
0 68	2		2	1 01	0	1	1
0.71	3		3	1 04	0	5	5
0.74	3		3	1 07	2	6	8
0 77	6	2	8	1 10	1	7	8
0.80	8	0	8	1.13		7	7
0.83	6	1	7	1.16		8	8
0 86	8		8	1.19		1	1
0 89	5		5				
0 92	3		3	Total	50	39	89
0.95	1		1	Mean	0.84	1.08	
0 98	2	1	3				

A somewhat lower percentage was obtained in the back cross of the same F_1 plants to the Red Pear variety (Table 3). Here tests were conducted in the same manner as in the preceding back cross (seedling progenies), and from a total of 89 progenies only six were of the crossover combinations, giving 6.7 per cent crossing over.

If these three back-cross generations are totaled, the average percentage crossing over becomes 10.9, there being 39 nonparental combinations in a total of 359 individuals in the experiment (Table 4). Accordingly, one may conclude that the ovate gene resides rather closely to the dwarf gene in the first chromosome.

TABLE 4.—*Summary of data showing intensity of linkage between fruit shape and tall-dwarf factor pairs*

Crosses	Tall		Dwarf		Percent-age cross-ing over
	Nonovate	Ovate	Nonovate	Ovate	
Back crosses:					
F_1 (DS×YP)×YP ♂	11	53	47	7	15 2
YP×F_1 (DS×YP)♂	6	67	70	9	9.9
F_1 (DS×YP)×Red Pear ♂	3	47	36	3	6.7
Total	20	167	153	19	10.9
F_2 generation:					
Dwarf Stone×Yellow Plum	72	39	49	0	
Expected with 10 per cent crossing over	80.4	39.6	39.6	0.4	

TABLE 5.—*Linkage relations between fruit shape and dwarf factors in F_2 if reciprocal crosses of Yellow Plum×Dwarf Stone*

Shape index	D	d	Total	Shape index	D	d	Total
0.77	1		1	1.16	10	3	13
0.80	0		0	1.19	12	8	20
0.83	5		5	1.22	5	5	10
0.86	6		6	1.25	4	11	15
0.89	5		5	1.28	2	5	7
0.92	6		6	1.31	1	2	3
0.95	7		7	1.34	0	6	6
0 98	5		5	1.37	2	3	5
1.01	4		4	1.40		1	1
1.04	6	1	7	1.43		1	1
1.07	8	2	10				
1.10	11	0	11	Total	111	49	160
1.13	11	1	12	Mean	1.08	1.25	1.13

Verification for this linkage is afforded by the F_2 generation of the Dwarf Stone $\times$ Yellow Plum cross (Table 5 and fig. 1). This gives the same "repulsion" phase of the linkage, and the summary shows 72 tall, nonovate; 39 tall, ovate; 49 dwarf, nonovate; 0 dwarf, ovate, among 160 plants. This fits a linkage hypothesis with 10 per cent crossing over fairly well. (See Table 4.) The absence of the double recessive class is to be expected, since at least 400 individuals are required with 10 per cent crossing over to give an even chance for this type to appear.

Further verification for this degree of linkage was obtained from this same F_2 generation by an F_3 test. In this case, 34 ovate (with shape index of 0.99 or less) tall F_2 plants were self-fertilized, and seedling progenies grown to determine the ratio of the DD and Dd genotypes. The results appear in Table 6, where there are 28 DD and 6 Dd types. With independent inheritance this ratio should be $1:2$, respectively. With 10 per cent crossing over the fit of observed and theoretical values becomes perfect.

TABLE 6.—*Showing linkage between fruit shape and tall, dwarf factor pairs in ovate, tall segregates from F_2 of Dwarf Stone $\times$ Yellow Plum cross, tested by F_3 seedling progenies*

Shape index	Homo-zygous tall prog-enies	Hetero-zygous progenies	Shape index	Homo-zygous tall prog-enies	Hetero-zygous progenies
0.77	1		0.98	2	2
0.80	0				
0.83	4	1	Total	28	6
0.86	5	1	Expected with 10 per cent crossing over	28	6
0.89	4	1			
0.92	6	0	Expected with no linkage	11	23
0.95	6	1			

Thus from four sources there is good evidence of a linkage between the recessive, ovate gene for fruit shape and the dwarf factor characteristic of the Dwarf Stone variety. The intensity of the linkage may be measured by an average value of 10 or 11 per cent crossing over.

LINKAGE OF THE OVATE AND PEACH GENES

Earlier work (*1, 3*) having demonstrated that the peach and dwarf characters, each being governed by a single recessive gene, were borne very closely together on the first chromosome of the tomato, it should of course follow that the ovate gene is linked with the peach factor. That this is true may be seen from the F_2 data in Table 7 (also in fig. 1) coming from a cross of the Red Peach (round-oblate) $\times$ Yellow Plum varieties.

TABLE 7.—*Linkage relations between fruit shape and peach factors in F_2 generations of the cross Red Peach$\times$ Yellow Plum*

Shape index	Field planting			Greenhouse planting		
	P	p	Total	P	p	Total
0.63	1		1			
0.68	2		2	2		2
0.73	7		7	3		3
0.78	9		9	2		2
0.83	4		4	6		6
0.88	6		6	1		1
0.93	17		17	3		3
0.98	24		24	16		16
1.03	13	5	18	6		6
1.08	7	6	13	7	3	10
1.13	3	8	11	2	4	6
1.18	1	1	2		6	6
1.23		0	0			
1.28		4	4			
Total	94	24	118	48	13	61
Mean	0.94	1.12		0 95	1.14	

Two plantings, one in the field and the other in the greenhouse, show a decided association of the ovate shape and P gene. No peach-type fruits with ovate shape were produced in a total of 179 F_2 individuals. Using the low point in the curve between the first and second modes (see fig. 1) as a separation point, the F_2 data of Table 7, might be grouped in a dihybrid distribution as follows:

$$106\ PO : 36\ Po : 37\ pO : 0\ po$$

This indicates an appreciable linkage between the two pairs of genes, and the intensity of the linkage could be determined. From previous experience with the ovate gene, however, the writer has become skeptical of any accurate linkage values calculated from F_2 data alone, particularly in the repulsion phase. MacArthur (5) gives values of 17 and 8 per cent crossing over between these genes (using the pyriform type). Experiments are now in progress, involving the three genes d–p–o in a three-point test that will give a better measure of this linkage, and will establish accurately the order of the genes in this first chromosome.

RELATION OF OVATE SHAPE TO FRUIT SIZE

That there is a distinct association between the ovate shape of tomato fruits and their size has already been pointed out (3). It has also been demonstrated that the first chromosome, bearing the dwarf and peach genes, carries major size factors governing fruit size or weight (3). Having located the ovate gene in this same chromosome, it becomes of considerable interest to present additional data on the genetic relations between this gene and fruit size.

Fruit size in this report is given by the average weight of the same number of fruits per plant as was used in determining the shape indices from the measurements of the polar and equatorial diameters. An average of eight mature fruits of normal character were weighed individually on balance scales, readings being taken to the nearest gram.

The simple correlations between fruit shape and fruit weight of the various crosses involved in this study were as follows:

F_2 — Yellow Plum × New Globe_____r=0. 54+0. 04
F_2 — Yellow Plum × Dwarf Stone_____r= . 56± . 04
F_2 — Yellow Plum × Red Peach_____r= . 61± . 04
F_1(YP × DS) × Yellow Plum_____r= . 45± . 03
F_1(YP × DS) × Red Pear_____r= . 47± . 06

It may be noted that in every case the correlation is high, positive, and statistically significant, indicating that ovate fruit shape is rather intimately associated with small size in these hybrid populations. In Tables 8 and 9 are included samples of these correlations, presented in order to give a visual demonstration of the relation between shape and size.

TABLE 8.—*Correlation table involving fruit shape and fruit weight in the F_2 generation of the Dwarf Stone × Yellow Plum cross*

Weight in grams \ Shape index	0. 75	0. 85	0. 95	1. 05	1. 15	1 25	1. 35	1. 45	Total
15		2			3	1	1		2
20	1	9	7	4	11	9	2		26
25		4	7	14	9	13	4		47
30			3	5	14	10	4	1	34
35		1			11	5	2	1	30
40							1		19
45						1			1
50									1
Total	1	16	17	23	48	39	14	2	160

r=0. 56±0. 04

TABLE 9.—*Correlation table involving fruit shape and fruit weight in the back-cross generation from F_1 (DS × YP) × YP and reciprocal*

Weight in grams \ Shape index	0 65	0. 75	0. 85	0. 95	1. 05	1. 15	1. 25	Total
10		1	5					6
15	1	14	32	11	23	5		86
20		6	39	19	31	27		122
25			8	4	16	16		44
30				1	5	9	2	17
35						1		1
Total	1	21	84	35	75	58	2	276

r=0. 45±0. 03

In all of these crosses involving the ovate shape the parental variety (Yellow Plum) has in each case contributed small size (or weight) together with the ovate shape. Accordingly, any linkage with size factors located on the first chromosome would ordinarily show a tendency for an association of large size and oblate shape as well as of small size and ovate shape. This association is the one so strongly exhibited by the five correlation coefficients listed above. There is, accordingly, some evidence of linkage between shape and size factors in these crosses.

⚊ The critical test, however, will be to demonstrate that the reverse correlation (large size associated with ovate shape) exists in certain crosses of large ovate by small oblate varieties. It seems, however, that there are no commercial, large-sized tomato varieties with distinctly ovate fruits. This may be a pointed suggestion that large size is not compatible with ovate shape from the morphological standpoint, in which case the correlation between ovate shape and size noted above is not due to genetic linkage. On the other hand, a genetic linkage of this sort may be the very thing that has prevented the plant breeder from producing such a type in times past. Search for large-fruited ovate types must now be made for a critical test of the size-shape correlation. As the evidence now stands, it is merely circumstantial in pointing to a genetic linkage between ovate shape and size, due to the location of the controlling factors in the same first chromosome of the tomato.

INHERITANCE OF ROUND AND OBLATE FRUIT SHAPES

Having determined that the ovate fruit shape in tomatoes may reasonably be determined by a single genetic factor, at least when acting in contrast both with the round (or roundish-oblate) and the oblate shapes, it becomes imperative to ascertain the interrelations of the round and oblate shapes themselves. It has been suggested that these various shapes of tomato fruits form a multiple allelomorphic system in their genetic behavior, a hypothesis that requires considerable verification.

Accordingly, data from a large series of crosses of round by oblate varieties have been assembled and will be discussed forthwith. The fruit shapes in these crosses were determined in a manner identical with those of the preceding crosses.

TABLE 10.—*Shape indices of crosses involving round and oblate fruit shapes*

Crosses	Oblate parent	Round parent	F_1	F_2	Grown
Bonny Best×Yellow Cherry	1.38	1.09	1.11	Figure 3	Greenhouse.
Golden Beauty×Red Cherry	1.50	1.06	1.15	Figure 4	Field.
New Globe×Yellow Cherry	1.29	1.07	1.09	Figure 5	Do.
Bonny Best×Yellow Peach	1.32	1.09	1.26	Figure 6	Do.
Dwarf Giant×Yellow Peach	1.35	1.09	1.27	Figure 7	Greenhouse.
Dwarf Stone×Yellow Peach			1.23	Figure 8	Do.

Six crosses involved in this investigation of the round-oblate situation have been arranged in Table 10, which gives the shape indices of the parents, F_1 and F_2 generations. In all cases samples of the three generations of plants in each cross were grown during the same season, and hence the indices are strictly comparable. The details of the F_2 and back-cross generations of each of these crosses are presented separately in Figures 3, 4, 5, 6, 7, and 8.

The six crosses in Table 10 may conveniently be grouped into two categories, first the crosses of the oblate by the round Cherry types and, second, oblate by the Peach (roundish-oblate) types. The Cherry varieties (Red and Yellow) bear fruits that are approximately round in shape, the average shape index being 1.07. The Yellow Peach variety is a trifle more oblate, its average index being 1.09.

There is a noticeable difference in the F_1 fruits of the two sets of crosses in the matter of dominance of size. Crosses of large oblate by small Cherry types give an F_1 plant with fruit showing a higher degree of dominance of small size ($\mathcal{Q}$) than is true in crosses of large oblate by the smaller Peach varieties. There is an analogous
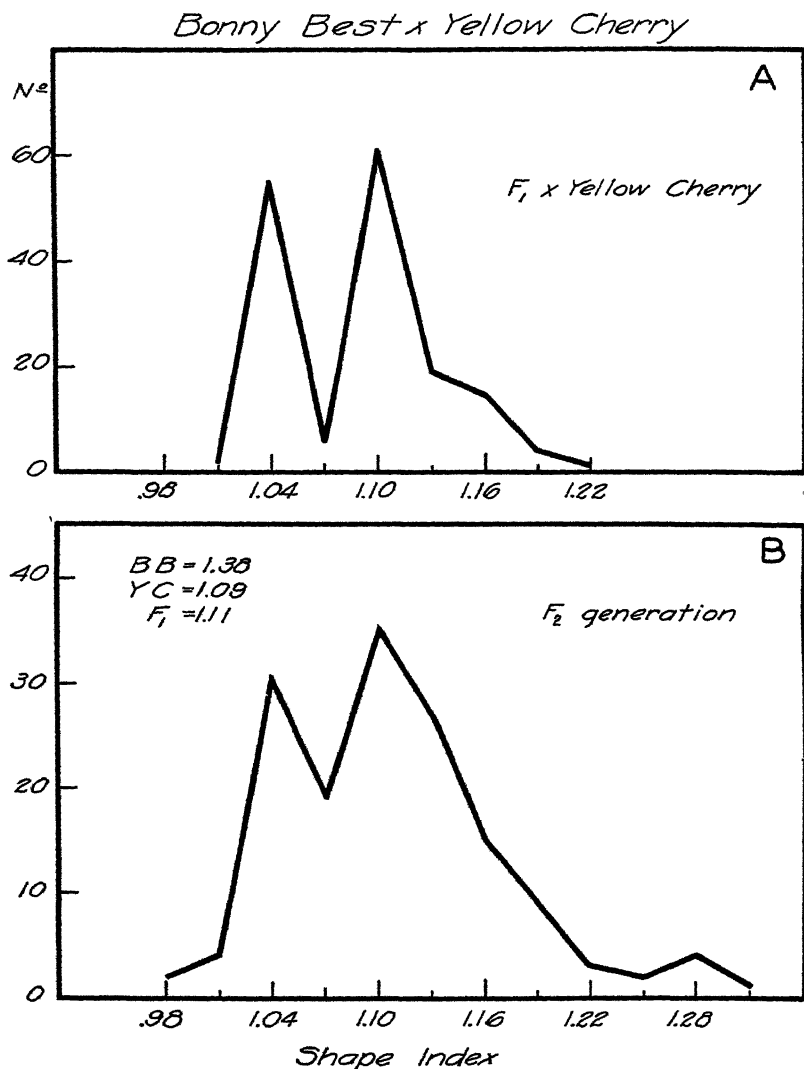
FIG. 3.—An F_2 and a back-cross generation of the Bonny Best (oblate)×Yellow Cherry (round) cross, showing distribution of fruit shape

behavior in the matter of fruit shape, the F_1 of the Peach-oblate crosses showing relatively more oblateness than the F_1 of the Cherry-oblate crosses (Table 10).

In Figure 3, showing the F_2 and back-cross generations of the Bonny Best (oblate) × Yellow Cherry (round) cross, there is definite evidence

of segregation of fruit shape. This is particularly true in the back-cross generation ($F_1 \times$ Yellow Cherry), which presents a sharp bimodal curve, indicating two major genotypes. A similar situation occurs in Figure 4, although here the differences are not so distinct.
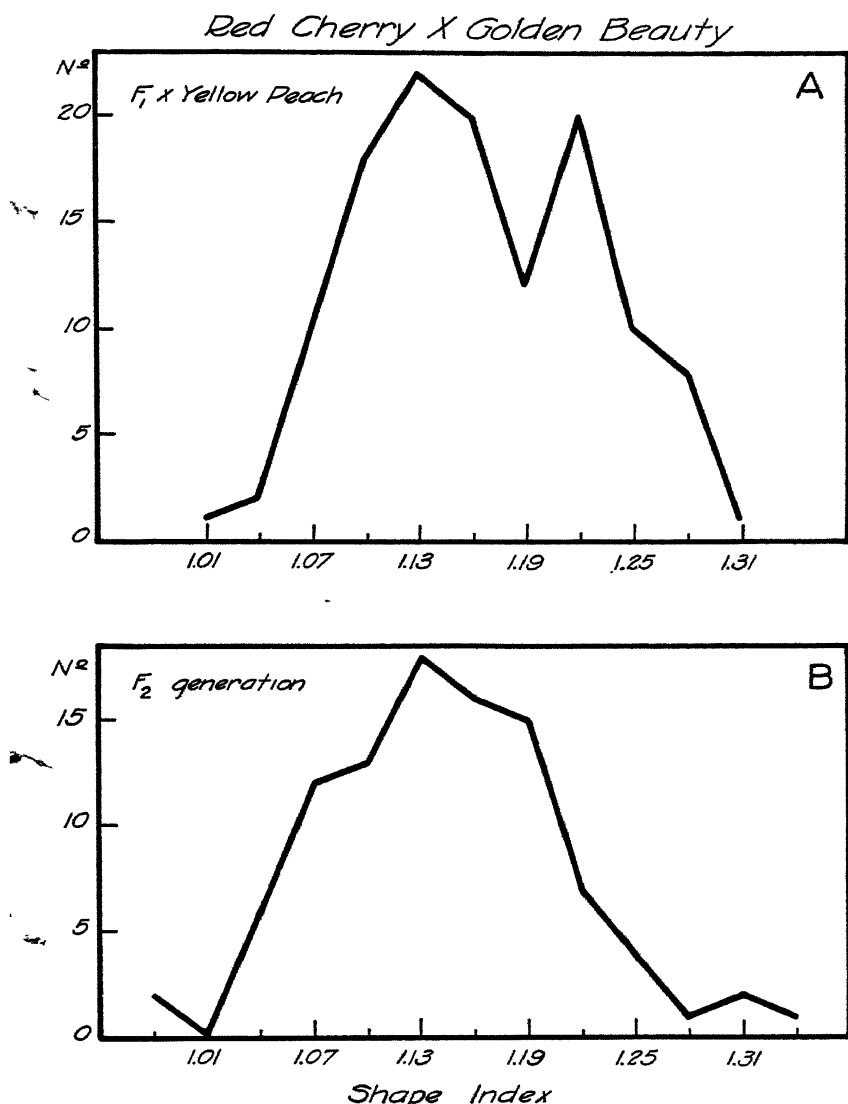
FIG. 4.—An F_2 and a back-cross generation of the Red Cherry (round)×Golden Beauty (oblate) cross, showing distribution of fruit shape

This is a cross of the Red Cherry × Golden Beauty (oblate) varieties. In this case the back cross was not made to a Cherry type but rather to the more roundish-oblate Yellow Peach variety. This might tend to decrease the influence of any two different genotypes to some extent. The third cross involving the Cherry type of fruit

in contrast with the oblate type (New Globe) appears in Figure 5.⅄. Here the F_2 generation exhibits a distinct trimodal condition. The back-cross generation to the very oblate type (Golden Beauty) shows but slight tendency toward bimodality. However, the number of

New Globe X Yellow Cherry

A

F_1 X Golden Beauty

B

$NG = 1.29$
$YC = 1.07$
$F_1 = 1.09$

F_2 generation

Shape Index
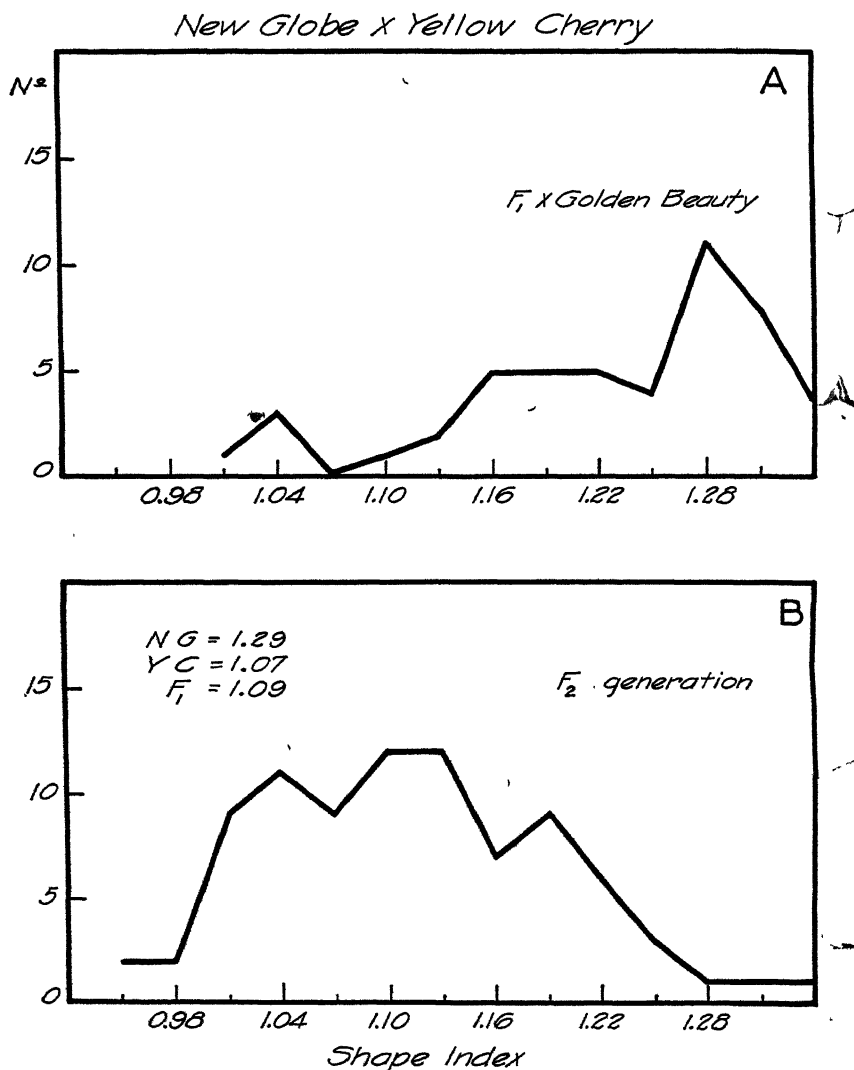
FIG. 5.—An F_2 and a back-cross generation of the New Globe (oblate)×Yellow Cherry (round) cross, showing distribution of fruit shape

plants in this back cross is really too small to offer evidence of any reliability.

Turning to the three crosses of the Yellow Peach variety (round to roundish-oblate) with the more oblate types, there is plotted in Figure 6 the first set of crosses. Here the F_2 generation (Bonny Best × Yellow Peach) exhibits some faint indications of segregation,

and the back-cross generation ($F_1 \times$ Yellow Peach) somewhat more evidence of it. Two fairly distinct modes are apparent, but they could not be classed as reliable indicators of simple genetical differences. There is better evidence in Figure 7, which shows the F_2 and back-
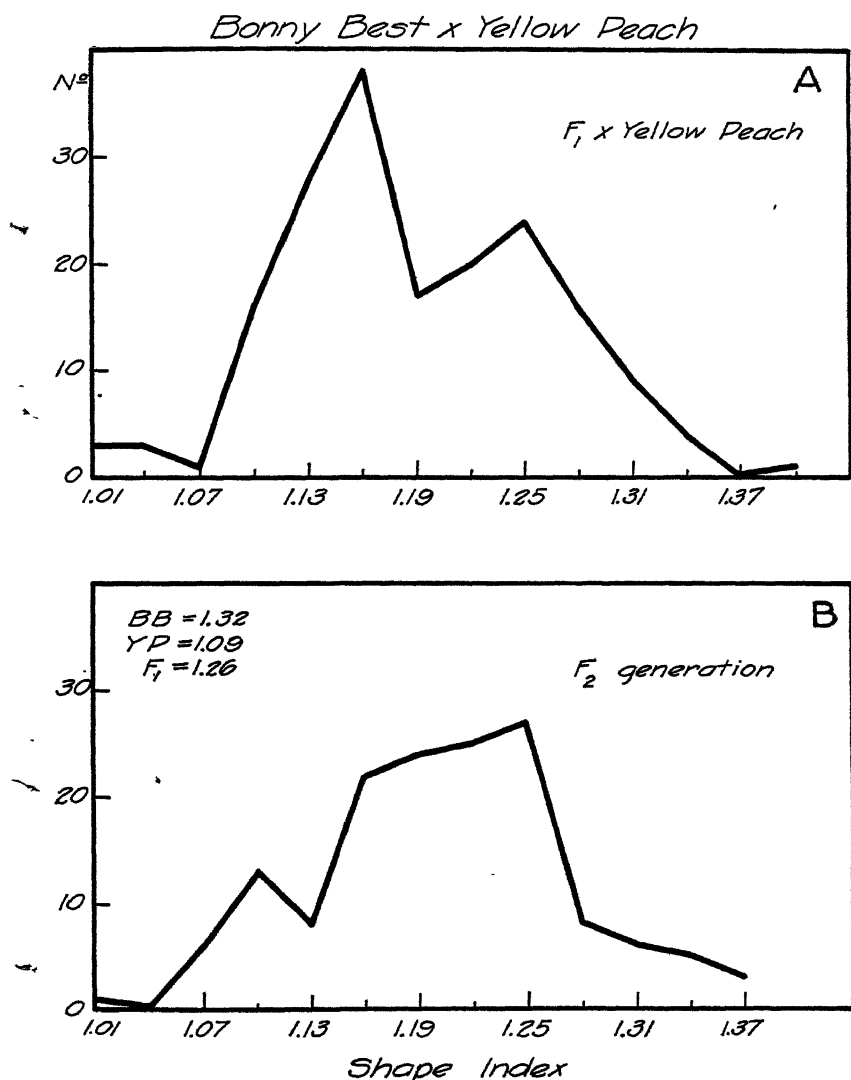
FIG. 6.—An F_2 and a back-cross generation of the Bonny Best (oblate)×Yellow Peach (round) cross, showing distribution of fruit shape

cross generations of the same Yellow Peach variety crossed with the oblate, Dwarf Giant. The back-cross generation especially is distinctly bimodal. In a similar cross, Dwarf Stone × Yellow Peach, shown in Figure 8, there is further verification of an analogous shape segregation in both F_2 and back-cross generations.

Thus in six different crosses of oblate by round types of fruit, there is some consistency in a tendency toward a simple segregation into oblate and round fruit shapes, modified, of course, by the inevitable agencies of unknown nature, mostly environmental per-
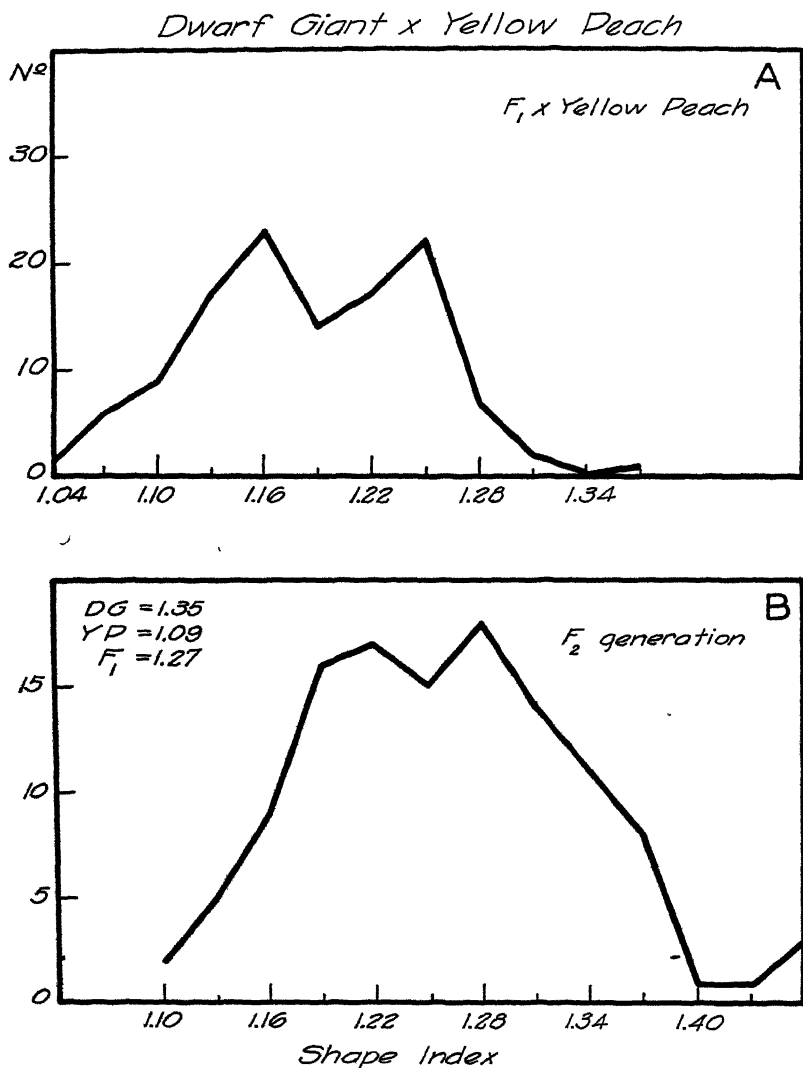
FIG. 7.—An F₂ and a back-cross generation of the Dwarf Giant (oblate)×Yellow Peach (round) cross, showing distribution of fruit shape

haps. True, in none of the crosses are the differences as marked as when the ovate shape of fruit is involved. Nevertheless, it is possible to predicate a genetic agency controlling fruit shape in the oblate-round end of the fruit-shape series that is analogous to the one discovered in the other (more recessive) end of the series. At least there seem to be major factors differentiating oblate and round shape.

A survey of the genetical relations of the oblate and round shapes of tomato fruits having suggested that it is possible for the difference between the two shapes to be attributed to a single major pair of genes, it becomes essential to consider if these genes are allelomorphic
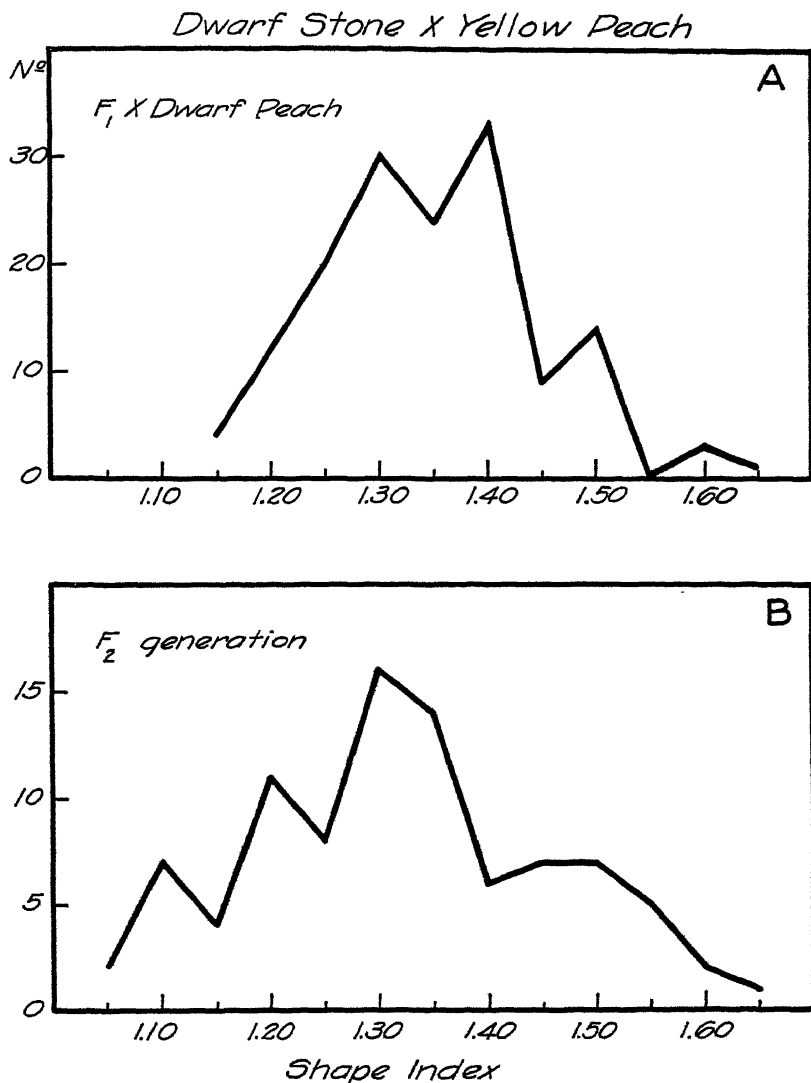
FIG. 8.—An F_2 and a back-cross generation of the Dwarf Stone (oblate)×Yellow Peach (round) cross, showing distribution of fruit shape

to the ovate genes. It will be recalled that the ovate gene has been proved to be a simple allelomorph to the oblate and also to the round-determining shape factors in crosses of oblate × ovate, and round × ovate types. This suggests strongly a multiple allelomorphic situation, but there is the other possibility that the oblate-round gene

differences discovered in the preceding crosses may be due entirely to a different set of genes than those allelomorphic to ovate shape.

TABLE 11.—*Shape indices in the F_2 and back-cross generations of the Bonny Best × Yellow Peach cross*

Shape index	F generation			F_1 × Yellow Peach		
	P	p	Total	P	p	Total
1.01	1	--------	1	--------	3	3
1.04	0	--------	0	--------	3	3
1.07	3	3	6	1	0	1
1.10	8	5	13	3	13	16
1.13	5	3	8	14	14	28
1.16	16	6	22	18	20	38
1.19	19	5	24	11	6	17
1.22	16	9	25	12	8	20
1.25	23	4	27	14	10	24
1.28	7	1	8	11	5	16
1.31	5	1	6	3	6	9
1.34	4	1	5	2	2	4
1.37	0	0	0	0	0	0
1.40	1	0	1	0	0	0
1.43	--------	1	1	0	0	0
1.46	--------	0	0	1	--------	1
1.49	--------	1	1	--------	--------	--------
Total	108	40	148	90	90	180
Mean	1.21	1.20	--------	1.20	1.18	--------
Closeness of fit	$P=0.94$			$P=0 20$		--------

TABLE 12.—*Shape indices of the F_2 generation of the Dwarf Giant × Yellow Peach cross*

Shape index	Stature		Fruit Pubescence		
	D	d	P	p	Total
1.10	2	--------	1	1	2
1.13	5	--------	3	2	5
1.16	9	--------	6	3	9
1.19	13	3	8	8	16
1.22	10	7	15	2	17
1.25	9	6	11	4	15
1.28	14	4	10	8	18
1.31	10	4	8	6	14
1.34	10	1	7	4	11
1.37	4	4	6	2	8
1.40	1	--------	1	--------	1
1.43	1	--------	1	--------	1
1.46	1	--------	1	--------	1
1.49	1	--------	1	--------	1
1.52	1	--------	1	--------	1
Total	91	29	80	40	120
Mean	1.26	1 27	1.26	1.25	--------

Data bearing on this point are assembled in Tables 11, 12, and 13, which also involve the dwarf (d) and the peach genes (p). These genes belong in the first linkage group, being closely linked with the ovate genes. If the major shape differences in the oblate × round crosses are allelomorphic to the ovate gene, linkage between shape and the peach, or shape and the dwarf characters, should be evident. And the intensity of the linkage should be identical with that of the ovate gene with these characters.

In Table 11, which shows the shape distribution for the smooth (P) and peach (p) type fruit separately in the Bonny Best × Yellow

Peach cross, there is no reliable proof of any distinct linkage between fruit shape and the *Pp* genes. In the F₂ generation the smooth (*P*) fruits average 1.21 and the peach (*p*) 1.20 in shape index. The latter should give a smaller index on the linkage hypothesis, but the actual difference is doubtfully significant. If the two F₂ distributions are compared to determine whether they may be samples of the same population, the probability is extremely high (*P* = 0.94) that they are identical. The same conclusion is apparent in the back-cross generation where the probability, while not so high (*P* = 0.20), is sufficiently so to indicate identity. Here also there is a faint suggestion, however, that the peach fruits have a smaller index (1.18 as compared with 1.20), but this difference also is too small for a linkage that should be as close as that shown in Table 7.

These conclusions are verified in the F₂ generation of the Dwarf Giant × Yellow Peach cross in Table 12, where the average shape indices of the smooth and peach types differ only by 0.01 (1.26 − 1.25). In this cross the tall-dwarf (*Dd*) genes also are involved, and they should exhibit the same linkage (or lack of it), since they are very closely linked with the *Pp* genes. Here the dwarf fruits have an index of 1.27 and the tall types an index of 1.26. A linkage relation should give a higher index for these dwarf segregates, but the difference found is again too small to mean a great deal.

TABLE 13.—*Shape indices in the F₂ and back-cross generations of the Dwarf Stone × Yellow Peach cross*

Shape index	F₂ generation					F₁ × Dwarf Peach				
	D	*d*	*P*	*p*	Total	*D*	*d*	*P*	*p*	Total
1.00	1		1		1					
1.05	1		1		1					
1.10	7		6	1	7					
1.15	3	1	3	1	4	2	2	1	3	4
1.20	10	1	10	1	11	8	4	4	8	12
1.25	7	1	5	3	8	8	12	10	10	20
1.30	14	2	12	4	16	14	16	16	14	30
1.35	11	3	9	5	14	11	13	13	11	24
1.40	6	0	4	2	6	17	16	17	16	33
1.45	6	1	6	1	7	5	4	4	5	9
1.50	6	1	4	3	7	10	4	4	10	14
1.55	5		3	2	5	0	0	0	0	0
1.60	2		2		2	2	1	1	2	3
1.65	1		1		1	1			1	1
Total	80	10	67	23	90	78	72	70	80	150
Mean	1.32	1.32	1.31	1.35		1.36	1.34	1.34	1.35	
Closeness of fit						*P* = 0.80		*P* = 0.81		

In Table 13, which gives the F₂ and back-cross generations of the Dwarf Stone × Yellow Peach cross, there is a similar lack of distinct evidence of any linkage between the *Dd* or the *Pp* genes and any factors controlling oblate and round fruit shapes in this cross. The closeness of fit has been determined in the back-cross generation, and the high values of *P* (0.80 and 0.81) show that the *D* and *d*, or the *P* and *p* distributions may be considered as samples of the same population. Accordingly, there is no marked evidence of any linkage of these genes and oblate or round fruit shape as the data are presented.

Before drawing such a conclusion, however, one very important point must be considered. In all of the crosses used as evidence (Tables 11, 12, 13) it has already been shown (*3*) that these genes *Dd* and *Pp* are very closely linked with a major factor for fruit size. It has also been demonstrated that in these very three crosses involving the Yellow Peach variety, the smaller Yellow Peach variety is carrying a major factor for large size, so that the peach segregates in these hybrid generations show an average fruit weight or size considerably in excess of the smooth fruited plants. This linkage will, of course, modify the shape relations in these three crosses, particularly in view of the high positive correlation between fruit shape and fruit size. Thus the peach types, which ordinarily should bear much the smaller shape index (because the Yellow Peach parent contributed the smaller shape index), show a shape index only a trifle smaller than the smooth fruits. Likewise there is so tight a linkage between these *Pp* genes and the *Dd* factors, that this would influence the dwarf segregates in a similar manner. Accordingly, any linkage between shape and size will be obscured by this complex situation. Here is a good example of how direct experimental evidence may be misleading at times if all pertinent facts are not taken into consideration.

Unfortunately, most of the writer's crosses involving oblate and round shapes and the *Dd* or *Pp* genes were in such combinations that the unusual linkage between *D* and *P* genes and size factors would obliterate the point in question. There was one cross, however, that did not involve the peach gene. This was a cross of the Dwarf Giant (oblate) × Yellow Cherry (round). In this case the dwarf gene was contributed by the parent with the oblate fruit shape. Only 77 F_2 plants of this cross were grown. Their fruit shape indices in relation to the *Dd* genes are arranged in Table 14.

TABLE 14.—*F_2 generation from the Dwarf Giant (oblate) × Yellow Cherry (round) cross, showing linkage between fruit shape and growth habit*

Shape index	D	d	Total	Shape index	D	d	Total
1.07	1		1	1.31	1	1	2
1.10	5		5	1.34		1	1
1.13	13		13	1.37		2	2
1.16	13		13	1.40		2	2
1.19	9	1	10				
1.22	16	3	19	Total	66	11	77
1.25	5	1	6	Mean	1.18	1.30	
1.28	3	0	3				

In this cross of an oblate and a round type there is a distinct association between oblate shape and the dwarf character, as well as between round shape and the tall or standard character. This would seem to indicate that the factors controlling the difference between the oblate and round fruit shapes in this cross were linked with the *Dd* genes. Crosses like this and including other varieties with similar shape differences should give critical evidence for the hypothesis that such shape genes are allelomorphic to the ovate shape gene; in other words, that the oblate, round, and ovate shapes are determined by multiple allelomorphs.

If further proof is adduced by means of such crosses, showing that the genetic difference between oblate and round shape is caused by a pair of genes that are linked with either the Dd or the Pp genes, the chain of evidence on the multiple allelomorphic nature of the shape factors will be complete. As the matter now stands the evidence is circumstantial, with but one line of direct proof in favor of the multiple allelomorphic system. Crosses have been made that should afford critical information on this problem in the near future.

Attention should be called to the significant correlation between shape and size in the series of oblate-round crosses, a situation that is surprisingly similar to that in the ovate-oblate crosses. There follows a list of simple correlations between fruit shape and fruit size:

F_2 — Bonny Best × Yellow Cherry_____ $r = 0.47 \pm 0.05$
F_2 — Golden Beauty × Red Cherry_____ $r =\ .45 \pm\ .05$
F_2 — New Globe × Yellow Cherry_____ $r =\ .66 \pm\ .04$
F_2 — Bonny Best × Yellow Peach_____ $r =\ .58 \pm\ .04$
F_2 — Dwarf Giant × Yellow Peach_____ $r =\ .52 \pm\ .05$
F_2 — Dwarf Stone × Yellow Peach_____ $r =\ .45 \pm\ .05$

In these crosses of oblate by round varieties the correlations of shape and size are approximately as high as were those in the hybrid generations of the ovate by oblate varieties. This certainly points to a genetic similarity between the three shapes, the oblate, the round, and the ovate, in their reaction to fruit size, and confirms the suspicion that the three form a multiple allelomorphic series.

DISCUSSION

Fruit shape in the tomato apparently is determined by a simpler genetic mechanism than is fruit size. In these investigations certain major genes for such fruit shapes as oblate, round, ovate (including pyriform) have been isolated by quantitative means, using careful measurements of the polar and equatorial diameters of the fruit. While the effect of these genes is modified by other agents to some extent, certainly they are not as subject to environmental influence as are fruit-size factors. The segregation of the various shapes in hybrid generations proved to be unexpectedly distinct. Whereas in crossing large by small fruited types, it has been impossible to recover the large parental size in F_2 generations of 200 to 300 plants, this has been done repeatedly with respect to shape characters. Ordinarily both parental shapes are recovered in F_2, indicating a much simpler genetic basis for these shape characters.

Experimental data demonstrate rather conclusively that ovate and round shapes as well as ovate and oblate shapes are allelomorphic on a simple monohybrid basis. Both F_2 and back-cross generations give evidence of this simple relation when shape is quantitatively determined. The recessive (both round and oblate being partially dominant) ovate gene is shown by critical tests to be linked with the dwarf gene, and accordingly belongs in the first linkage group of the tomato, being removed from the dwarf gene by approximately 10 or 11 units. It is also linked with the first chromosome peach gene. This means that these allelomorphs of the ovate gene, namely, the oblate and the round determining factors, all reside in the first chromosome.

Evidence is presented to show that a very high positive correlation exists between shape and fruit size in crosses of large oblate × small ovate, or large round × small ovate types. This may be taken to mean that the ovate gene (and its allelomorphs) is closely linked with a major factor for fruit size located in the first chromosome. Previous experiments have proved that a major size factor is present in the first linkage group, there being good evidence of linked inheritance between fruit size and the *Dd* and *Pp* genes (*3*). The association of fruit size and the ovate gene seems to be corroborative testimony to the existence of definite size factors in the chromosomes of the tomato. There remains, however, the alternative explanation that the association of shape and size may be traceable to a morphological relation between the two characters in the cell growth of the tomato group, and that the size factors are in reality the result of the shape determiners.

Limited observations have shown that the pyriform shape acts genetically like the ovate shape in crosses involving the two forms. The genetic difference between the ovate and pear shape is not clearly established as yet, but it is known that the latter is incompletely recessive. There is no evidence that the gene determining pyriform shape belongs in the first linkage group.

Dominance in fruit shape, while existent, is far from complete. There seems to be only a slight dominance of the more oblate types. Intermediate shapes in crosses of these various forms are very conspicuous. The ovate or pyriform shapes disappear in the F_1 fruits when crossed with either round or oblate types. Crosses of oblate by round forms show very little dominance of the former. Thus in the series of fruit shapes noted above there seem to be differential degrees of dominance, traceable no doubt to the nature of cell growth and development in the maturing fruit.

While the hypothesis establishing a multiple allelomorphic system for the oblate-round-ovate series of fruit shapes has been developed from some rather tangible experimental data, it needs considerable verification, particularly with varieties other than those used herein. The decks are now cleared for action on this suggestive hypothesis, and the weak points in the situation need critical attention. Warren's (*6*) hypothesis of complementary action of two pairs of shape factors possibly is not in agreement with this new assumption. However, careful scrutiny of Warren's experimental data fails to demonstrate any adequate proof of his hypothesis. Where he expects an F_2 ratio of 9 "deep" to 7 "shallow" fruits, his data on six F_2 distributions are very unsatisfactory. Whereas the total of these six progenies agrees fairly well with a 9 : 7 ratio, the individual progenies show great variation, two of the six having deviations of more than three times their probable errors. What is more to the point, however, is the fact that the critical back crosses which should verify his hypothesis are all dismissed with the statement that great difficulty was encountered in classifying the fruits (by inspection), there being certain influences (fasciation) that interfered with the determination of shape. The complementary factorial hypothesis for fruit shape accordingly rests on an insecure basis.

If the suggestion of a multiple allelomorphic series involved in fruit shape should receive the necessary confirmation, it is urged that the symbolism for the genetic factors be considered as soon as possible

so as to avoid unnecessary confusion. Instead of the biliteral *pr* symbol, the basic gene symbols *Oo* (oblate-ovate) are suggested. The various allelomorphs may then be designated by priming the upper and lower case letters, or by giving them a descriptive super-script, as, for example: O = oblate; o^r = round; o = ovate (plum).

SUMMARY

By means of quantitative determinations of fruit shape (using as a shape index the ratio of the equatorial to the polar diameter) in the tomato, it is established that ovate fruit shape is determined geneti-cally by a major, recessive gene. Back-cross data particularly demonstrate this fact. The dominant (incompletely) allelomorphs of the ovate shape were the oblate or the round shapes of the fruit.

Linkage determinations prove that this ovate-determining gene resides in the first chromosome of the tomato. Various linkage tests show that the dwarf and ovate genes are closely linked, there being approximately 10 or 11 per cent crossing over. The peach gene also is proved to be linked with the ovate-shape gene.

High, positive correlation is demonstrated between shape and size of tomato fruits in a series of F_2 and back-cross generations involving the various shapes. This may mean that fruit-shape factors of the first chromosome are linked with major fruit-size factors.

Crosses of oblate by round types give evidence of the action of single, major genes for these fruit shapes, although the differences are not so distinct as those differentiating ovate from either round or oblate shapes. Environmental agencies seem to affect oblate shape more than ovate shape. High correlations between shape and size were also found in the F_2 of these crosses.

Crosses involving oblate, round, and ovate shapes, having demon-strated that these three fruit shapes are determined by certain major genes residing in the first chromosome, the hypothesis is offered that the three form a multiple allelomorphic system. The order of domi-nance in this series is, oblate, round, and ovate.

There are many indications that fruit shape in general is deter-mined by a simpler genetic mechanism than is the case with fruit size.

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THE GOSSYPOL CONTENT AND CHEMICAL COMPOSITION OF COTTONSEEDS DURING CERTAIN PERIODS OF DEVELOPMENT [1]

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INTRODUCTION

The recent findings of Schwartze and Alsberg (7),[3] showing that the gossypol content of cottonseeds tends to vary with the locality in which the seeds are grown, and is increased in those seeds which have a high oil content, has made it desirable to determine at what stage of maturity the maximum amount of gossypol is present. The writer desired to know if the gossypol accumulated in the seeds at the same time that there was an accumulation of other materials such as oil and protein, or if it formed after the seeds had fully matured and had separated from the plant. Ever since the isolation of gossypol as a toxic constituent of cottonseeds (10) the writer and coworkers at this station have been interested in the factors which determine the gossypol content of cottonseed meal and have recently published some of the results of this work (4, 5). For obvious reasons, the gossypol content will depend primarily upon the gossypol contained in the original seeds. It is the writer's opinion that climatic or weather conditions are important factors to be considered and that such conditions might be more or less effective, depending upon the time of planting and perhaps of gathering the cotton. The amount of rainfall during the growing period of the cotton plant governs to some extent the amount of oil in the seeds, and if it can be demonstrated that the gossypol content is regulated by the formation of oil, then these same conditions will in turn affect the gossypol content. Present knowledge of the gossypol content of cottonseeds as dependent upon variety, oil content, and geographical region of growth of the seeds is confined to the publication cited above (7).

Balls (2) working with Egyptian cotton No. 77 finds that the cottonseed has reached its full size between 18 and 21 days after the flowers have opened, the boll reaching its full size soon after, but that the seed continues to develop for some time. After 45 days the boll begins to crack and the cotton is usually ready to be picked within 5 or 6 days thereafter. Garner, Allard, and Foubert (6) studying the oil content of cottonseeds at different stages of maturity, found that the increase of oil proceeded somewhat more rapidly than the growth of the seed. In experiments with soy beans these investigators found that during the final stages of ripening there

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Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 10
May 15, 1927
Key No. Okla.–8

(987)

was a slight decrease both in the size of the seed and in the oil content. This phenomenon they attributed to continued respiratory activity after assimilation had ceased. Referring to the transformation of carbohydrate to fat they state that "under proper conditions this transformation takes place in unripe seeds detached from the mother plant, further indicating that the oil is derived from the carbohydrates."

Since gossypol contains only carbon, hydrogen, and oxygen, and is closely associated with the oil in cottonseeds in other ways, it appeared possible that it might also be formed at the same time and that it might be associated in some way with the formation of the oil. The practical value of any such finding is perhaps obscure until our very limited knowledge of the formation of gossypol and its properties, except as these properties are manifested in the feeding of cottonseed products to livestock, is considered. Concerning the rôle of gossypol in the life of the cotton plant little is known. Carruth (3) speaks of gossypol as being formed by the disintegration of adjacent cells. In the light of the histological studies upon the glands of the cotton plant by Stanford and Viehoever (9), this statement is well founded.

EXPERIMENTAL MATERIAL AND METHODS

COLLECTING SAMPLES

Cottonseeds of a selected variety, Oklahoma Triumph 44, were used in this study. They were obtained from the field plots in which cotton variety tests were being made by the department of agronomy. The cotton crop, grown on upland soil, was planted May 10, and picking began October 15. There was a moderate amount of rainfall throughout the growing season, and the season was favorable for a large cotton crop. On October 1 bolls at different stages of development as determined by their appearance and condition, were collected in the field and brought to the laboratory for an analysis of the seeds. In this collection were full-size bolls approximately 50 days old, and showing signs of cracking, bolls which had just opened; bolls which had been open for 2 or 3 days; those which had been open for 5 or 6 days; and finally, bolls which had been open for longer periods and from which the cotton was hanging down, the locks having lost much of their firmness.

On February 1 another collection of matured seeds was made from the unpicked cotton left in the field, and at the same time a representative sample of seeds from the whole field was obtained from the cotton gin. All the seeds were removed from the bolls by hand and delinted with sulphuric acid. The immature seeds were dried at a temperature sufficiently low to prevent the destruction of gossypol or its change to a less soluble form. The seeds were then ground separately and sampled for chemical analysis, including gossypol determinations. Representative samples of the unground seeds were reserved for determinations of their total dry matter.

The methods of analysis used by the Association of Official Agricultural Chemists (1) were employed for the chemical determinations, anhydrous diethyl ether being used as an extractive for the fat determinations. The gossypol was determined by Carruth's method as modified by Schwartze and Alsberg (7). Fifty-gram samples were used and the seeds were extracted for 24 hours in a Soxhlet extraction

apparatus. Calculation of the gossypol in the aniline gossypol precipitates was made by use of the conversion factor .74, since the composition of this precipitate approaches $C_{30}H_{28}O_92C_6H_5NH_2$, and this factor has been used by previous investigators (*8*).

DISCUSSION AND RESULTS

PROXIMATE ANALYSIS

The results of the several analyses are given in Table 1.

TABLE 1.—*Chemical composition and gossypol content of cottonseeds at different stages of maturity*

Sample No.	Condition of boll or seeds	Composition of seeds on dry-matter basis					
		Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Gossypol
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	About to open	6.20	28.46	18.56	32.81	13.97	0.045
2	Just open	3.93	31.11	14.46	26.49	24.01	.425
3	Open 2 to 3 days	3.16	28.46	15.13	28.43	24.82	.461
4	Open 5 to 6 days	3.15	29.86	15.33	27.93	23.73	.538
5	Open over 6 days	3.33	28.37	15.20	28.69	24.41	.546
7	Mature seeds picked Feb. 1	3.24	30.29	14.37	26.34	25.76	.551
6	Seeds obtained from gin	3.34	29.01	15.78	28.46	23.41	.452

From these results it would seem that there is a period of intense oil formation occurring about the time the boll begins to crack and just previous to its opening. This wide difference in the oil content of the seeds picked at these two intervals appeared questionable, and another picking of the unopened bolls was made. The oil content of the seeds obtained in this second picking, 15.05 per cent, was only slightly higher than the one shown in Table 1, which satisfied the writer that the first figures obtained were approximately correct. Other factors not considered here may influence the oil content of the seeds at this stage; but as this study was concerned primarily with gossypol determinations, the influence of these factors was not investigated further. However, it is interesting to note that there is a slight but variable increase in the oil content of the more mature seeds and that this amount did not show a decrease even when the seeds were allowed to remain in the field for several months after they had matured. The relative amount of oil in the seeds was nearly doubled between the first and last stages of development.

The protein content of the immature and mature seeds does not show any marked increase as the seeds develop. This indicates what might be expected, namely, an early deposition of nitrogenous material. There are, however, fluctuations between the protein content of seeds picked at different times which may be accounted for by the variable size of the seeds and by errors introduced in obtaining samples. The same conditions hold true for the results of the fat determinations, although an attempt was made to eliminate these errors as far as possible by obtaining samples from a selected area of the field. At a later date the writer hopes to be able to make a more thorough study of the accumulation of both oil and protein during the successive stages of development of the cottonseed.

The ash, crude fiber, and nitrogen-free extract of seeds from opened bolls were considerably less than from unopened bolls, but remained quite constant for the seeds picked during the later stages of development. These general results are in harmony with the findings of previous investigators working with other kinds of seeds as well as with the results of some unpublished work in this laboratory by N. B. Guerrant.

GOSSYPOL DETERMINATIONS

The gossypol determinations on seeds at various stages of maturity given in Table 1 showed some very interesting results. The gossypol content of the mature seeds was much higher than the writer has found for other samples grown in this locality, which were of no particular variety and had been in storage for some time. The formation of gossypol proceeded much more rapidly than that of oil, the gossypol making its greatest increase during the first two stages and continuing to increase at a very slow rate during the later stages. Although there are some indications that gossypol formation is concurrent with the formation of oil, there seems to be no direct relationship between the two; and in the case of sample No. 4, which showed a relatively lower oil content, the gossypol content was increased. The high gossypol content of those seeds which had matured and were left standing in the field is perhaps more indicative of the lysigenous origin of gossypol.

WEIGHT OF COTTONSEED IN RELATION TO ITS CHEMICAL COMPOSITION AND GOSSYPOL CONTENT

In order to observe the absolute as well as the relative changes in the oil content of cottonseeds collected at two stages of maturity, Garner, Allard, and Foubert (*6*) determined the weight of the seed and from the weight calculated the grams of oil in 1,000 seeds. Although the changes occurring in the weight of the seeds do not necessarily represent the exact growth made by the seeds between the stated intervals, it was felt that to proceed on such an assumption might aid in interpreting the data obtained in this study.

The dry weight of 500 seeds from several pickings was determined and the analysis of these calculated from the figures in Table 1. From these results, which are given in Table 2, it is evident that there was considerable growth between the first two stages, resulting in increased amounts of all the constituents.

TABLE 2.—*Chemical composition and gossypol content of 500 cottonseeds at different stages of development*

Sample No.	Condition of boll or seeds	Weight of dry seeds	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Gossypol
		Grams	Grams	Grams	Grams	Grams	Grams	Grams
1	About to open	20.09	1.25	5.72	3.73	6.59	2.81	0.010
2	Just open	38.46	1.51	11.96	5.56	10.20	9.23	.165
3	Open 2 to 3 days	43.27	1.37	12.32	6.55	12.30	10.74	.200
4	Open 5 to 6 days	44.77	1.41	13.36	6.86	12.51	10.62	.241
5	Open over 6 days	40.63	1.35	11.53	6.18	11.65	9.92	.222
7	Mature seeds picked Feb. 1	40.32	1.31	12.25	5.80	10.58	10.38	.222
6	Seeds obtained from gin	40.96	1.36	11.88	6.46	11.66	9.60	.15

A comparison of Tables 1 and 2 serves to emphasize some important differences in the manner of presenting data of this kind.

From Table 1 it appears that there is a decrease in the crude fiber, ash, and nitrogen-free extract of cottonseeds as they mature, while Table 2 shows an increase in the constituents. Both tables show an increase in the amounts of the other constituents which represent the greatest changes taking place in the seeds as they develop up to the time that the boll has been open for several days. Following this period the results are more or less irregular. The writer believes Table 2 to be a more correct interpretation of what actually happens in the seeds and has used the figures given in this table as a basis for drawing his conclusions. No explanation is offered for the large difference in weight between the seeds in samples Nos. 4 and 5 at this time, although a slight decrease in weight may be expected, due possibly to respiration. The gossypol content of the seeds is shown to be quite independent of their weight, but is slightly increased with the age of the seeds; and these facts lead the writer to think that storage conditions may also have an effect upon this amount.

SUMMARY

The gossypol content and chemical analyses of cottonseeds of the variety Oklahoma Triumph 44, picked at different stages of development, are reported. The seeds were obtained at the different stages from the time the boll was ready to open until it had fully opened, and also included those seeds which had remained in the field unpicked for several months. The greatest change in the composition of the seeds over the range studied occurred at the time the boll was mature and about to crack, from which time until the boll opened the gossypol content increased rapidly and continued to increase for some time thereafter. This increase in gossypol was greater than the increase of any of the other constituents, all of which showed only small increases after the boll had opened. No correlation was found between the formation of oil and the formation of gossypol.

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VARIANTS IN USTILAGO NUDA AND CERTAIN HOST RELATIONSHIPS[1]

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INTRODUCTION

Previous to the investigations made by Tisdale and Tapke [2] it was thought that infection of barley by loose smut, *Ustilago nuda*, took place only through the young embryo at flowering time. These investigators found that certain varieties of barley became heavily infected by loose smut if dehulled seed was inoculated with spores of the fungus before sowing. Low percentages of infection were obtained when hulled seed was inoculated. The fact that seed-surface disinfectants control loose smut in some varieties, especially the six-rowed varieties, indicates that considerable infection may take place from seed-borne spores.[3][4] Surface disinfectants, however, do not prove satisfactory in many cases, which indicates that considerable floral infection also takes place. In an unpublished report (1923) by R. S. Kirby, formerly extension pathologist of the New York Agricultural Experiment Station, it is stated that surface disinfectants, such as formaldehyde and the organic mercury compounds, control loose smut in six-rowed barleys but fail to control it in Alpha, a two-rowed barley grown extensively in New York. A news item furnished by the local farm bureau and published by the Sun of Norwich, N. Y., April 17, 1925, stated that surface disinfectants controlled loose smut in the six-rowed winter barleys but not in Alpha. The cause of these differences was not known. Climatic factors, morphological and physiological characters of the host, and variants or physiologic strains of the fungus were among the possible explanations. Investigations were started in the fall of 1924 in an attempt to answer some of the questions covering the action of loose smut on barley. The results of these investigations are reported herein.

METHODS AND MATERIALS

All seed for these experiments was obtained from smut-free plots of pure varieties of barley. Following methods previously used,[5] the

sₐₑₐs were carefully dehulled before inoculation. The dehulled seeds were thoroughly shaken in envelopes or vials with sufficient spore material to render them dark in color. To prevent the mixing in the greenhouse of smut collections obtained from various sources, the smutted heads were inclosed in glassine bags. In collecting the smutted heads the stems were cut just below the bags. As soon as the smut was dry the spores were carefully rubbed from the heads inside without opening the bags. The outside of the bag was then thoroughly washed with a cloth wet with bichloride of mercury (1 : 1,000), and the spores placed in small bottles which were kept corked until it was time to inoculate the seed, which was done just before sowing. The seeds were carefully spaced 1½ or 2 inches apart in rows 6 inches apart on benches in a greenhouse at Arlington Experiment Farm, Rosslyn, Va. In some cases smut injured germination and weakened the seedlings to such an extent that it was necessary to break the surface of the soil and straighten the abnormal seedlings so that they could develop.

EFFECTS OF SMUT ON GERMINATION AND GROWTH

A description of the injury to germination and seedling development by *Ustilago nuda* has been given by Tisdale and Tapke.[6] This same type of injury was observed again in the present investigations. It was noted also that in 1924–25 fewer of the seedlings from inoculated seed grew to maturity than did those from uninoculated seed. Table 1 shows the numbers of seedlings from uninoculated and inoculated seed two weeks after sowing, the number of plants which matured from these seedlings, and the percentages of smutted plants for each variety tested.

Table 1 shows that the germination of the seed of the Nakano Wase variety was injured by the inoculation to a greater extent than that of any other variety, although with one exception no smut occurred in the mature plants. A high percentage of plants from inoculated seed of both Nakano Wase and Orel died before maturity, regardless of the appearance of any smut in the heads of plants which matured. Every variety tested showed some weakness as the result of seed inoculation.

Similar results were obtained in experiments in the winter of 1925–26, in which a larger number of smut collections were used. The effect of these various collections of smut on Tennessee Winter and Hannchen barleys, the only varieties on which all the smuts were used, is shown in Table 2. Tennessee Winter showed the most injury and Hannchen the least injury of all the varieties tested in this experiment.

[6] TISDALE, W. H., and TAPKE, V. F. INFECTION OF BARLEY BY USTILAGO NUDA THROUGH SEED INOCULATION. Jour. Agr. Research (1924) 29: 263–284, illus. 1925.

TABLE 1.—*Effect of inoculating dehulled seed of six different varieties of barley with spores of loose smut obtained from different sources*

[Test made in greenhouse at Arlington Experiment Farm, Rosslyn, Va. Seed planted November 20, 1924]

Variety and locality from which inoculum was obtained	Varieties inoculated, number of seedlings on Dec. 4, number of plants maturing, and percentage of smutted plants								
	Han River (C. I. 206)			Hannchen (C. I. 531)			Nakano Wase (C. I. 2166)		
	Number of seedlings	Number of mature plants	Percentage of smutted plants	Number of seedlings	Number of mature plants	Percentage of smutted plants	Number of seedlings	Number of mature plants	Percentage of smutted plants
	50 seeds			*50 seeds*			*50 seeds*		
Control [a]	48	43	0	46	39	0	42	39	0
Alaska (Rosslyn, Va.) [a]	45	37	16.2	33	28	0	20	13	7.7
Han River (Rosslyn, Va.) [a]	46	36	30.6	46	47	100.0	39	25	0
Unknown (Egypt) [b]	46	39	0	48	43	2.3	39	27	0
Manchuria (Madison, Wis.) [a]	44	28	7.1	40	33	0	24	20	
Tennessee Winter (Rosslyn, Va.) [b]									

Variety and locality from which inoculum was obtained	Varieties inoculated, number of seedlings on Dec. 4, number of plants maturing, and percentage of smutted plants								
	Orel (C. I. 351)			Tennessee Winter (Selection 52)			C. I. 2222 (hullless)		
	Number of seedlings	Number of mature plants	Percentage of smutted plants	Number of seedlings	Number of mature plants	Percentage of smutted plants	Number of seedlings	Number of mature plants	Percentage of smutted plants
	50 seeds			*50 seeds*			*25 seeds*		
Control [a]	35	33	0	43	24	4.2	19	20	0
				100 seeds					
Alaska (Rosslyn, Va.) [a]	33	11	100.0	78	41	80.5	19	17	0
Han River (Rosslyn, Va.) [a]	35	21	85.7	80	51	82.4	20	15	6.7
Unknown (Egypt) [b]	38	21	0	84	58	8.6	19	19	0
Manchuria (Madison, Wis.) [a]	31	17	88.2	70	37	81.1	20	18	5.6
				50 seeds					
Tennessee Winter (Rosslyn, Va.) [b]				42	25	64.0			

[a] Smut obtained in 1924.   [b] Smut obtained in 1923.

TABLE 2.—*Effect of inoculation with spores of loose smut on the germination of 40 dehulled seeds each of Tennessee Winter and Hannchen barleys, sown November 24, 1925, in the greenhouse at Arlington Farm, Rosslyn, Va.*

| Smut lot No. | Number of seeds germinating on different dates and final percentages of smutted plants | | | | | | | | | | | | |
| | Tennessee Winter (selection 52) | | | | | | Hannchen (C. I. 531) | | | | | | |
	Dec. 1	Dec. 2	Dec. 3	Dec. 4	Jan 6	Percentage of plants smutted	Nov. 30	Dec. 1	Dec. 2	Dec. 3	Dec. 4	Dec. 19	Percentage of plants smutted
Control	27	31	34	34	35	0	22	37	39	39	39	40	0
1	16	26	27	28	36	0	18	30	34	34	33	40	0
2	9	10	16	17	32	85.7	12	33	38	38	38	40	0
3	8	21	22	28	34	72.7	15	35	39	39	40	40	72.5
4	20	28	32	33	37	0	19	30	32	34	34	37	0
5	4	10	11	13	29	78.6	14	30	37	37	37	38	97.4
6	2	11	12	10	22	68.2	12	26	31	34	32	34	97.0
7	7	13	17	17	34	93.6	4	17	27	29	30	39	0
8	4	14	16	18	35	71.4	8	31	39	40	40	40	100.0
9	11	24	28	29	36	81.8	8	30	36	37	37	39	0
10	5	9	14	14	33	75.8	5	31	38	40	40	40	95.0
11	2	10	14	15	32	75.9	13	31	36	37	37	36	91.7
12	19	28	31	32	35	0	23	37	37	37	37	40	0
13	21	29	30	30	36	0	27	38	39	39	39	39	0
14	7	15	16	16	26	84.0	18	36	37	39	38	40	0
15	21	28	29	30	34	0	28	38	40	40	40	40	0
16	9	19	21	22	35	77.8	26	39	40	40	40	40	0
Control	25	32	34	35	40	0	34	40	40	40	40	40	0
17	8	18	23	23	34	66.7	15	35	37	37	37	39	92.3
18	15	28	32	33	35	0	27	35	37	37	37	37	0
19	5	15	22	22	28	96.0	16	35	37	37	37	39	0
20	6	19	23	23	32	96.8	19	36	37	38	38	40	0
21	5	21	25	27	32	93.6	16	37	38	38	38	38	0
22	12	20	25	25	33	90.9	28	36	37	38	38	40	0
23	7	15	18	19	33	72.4	18	35	37	38	38	39	0
24	6	15	17	16	29	74.1	23	31	33	33	34	38	0
25	6	15	19	20	32	81.3	23	35	36	37	38	39	0
26	6	16	19	20	30	86.2	29	36	36	37	37	38	0
27	5	15	20	19	34	90.3	28	35	37	38	38	40	0
28	8	19	24	24	32	92.9	29	35	37	37	37	a 39	2.6
29	12	19	26	28	31	93.1	31	38	38	38	38	a 40	0
30	6	14	19	21	34	90.6	30	37	37	37	37	a 40	0
31	5	17	22	23	30	85.7	31	36	37	37	37	a 39	0
32	19	31	31	31	35	2.9	30	32	32	33	33	a 35	5.7
Control	31	33	34	35	37	0	39	39	40	40	40	a 40	0

a Record made Dec. 18.

In smut lots Nos. 1, 4, 12, 13, and 15 germination of Tennessee Winter was retarded less than the others. Even these lots, however, showed some retardation as compared with the controls. The plants from the control rows were smut free. In all other smut lots germination and development of the plants were affected. Hannchen was injured less than Tennessee Winter. Some of the most severe injury to germination occurred in smut lots which produced no smutted heads in the mature plants. Smut lot No. 7 is an example. This collection showed a marked effect on germination and the early stages of seedling development in Hannchen, but heads of the mature plants were smut free. The same smut, however, produced over 93 per cent of smutted plants in Tennessee Winter. Plants from inoculated seed which failed to become smutted were cut back near the surface of the soil after the smut records were made. A few of them produced secondary culms. Three plants of Han River and one of Tennessee Winter produced smutted heads in th secondary growth. In view of these results it is obvious that the term "infection" can not be used accurately to include only the

appearance of smutted heads in mature plants. Plants may be heavily infected and show no smut at maturity. The effect of the fungus on germination and plant development may prove of value also in studying strain differences in loose smut of barley.

SMUT STRAINS AS INDICATED BY PERCENTAGES OF SMUTTED PLANTS

In addition to the information on germination and plant development, Table 1 gives the percentages of smutted plants. One item of interest shown in this table is the occurrence of 100 per cent of loose smut in Hannchen grown from seed inoculated with smut spores from Han River, while plants of the same variety grown from seed inoculated with spores from Alaska were smut free. Of further interest, however, is the fact that Alaska smut spores produced high percentages of the disease in other varieties. Both the Alaska and Han River plants, from which the inoculum for Hannchen was collected, were grown in the greenhouse from dehulled seed inoculated with spores taken from the same collection of Tennessee Winter smɯ t in 1923. There seemed to be two possible explanations for the occurrence or absence of smut in Hannchen, either one or both of which may be concerned. The first and apparently the more probable explanation is that two strains of smut occurred in the original collection from Tennessee Winter barley, one producing smut in Alaska but not in Hannchen and the other producing smut in boɯ Hannchen and Han River but not in Alaska. The second is that there was only one strain of the smut in the original collection and that the fungus was influenced by Alaska in some way which prevented it from producing smut in Hannchen. Further data bearing on these points were obtained in a later experiment. The smut from Alaska produced 100 per cent of smut in Orel. Likewise the spores collected from Manchuria barley at Madison, Wis., in 1924 (Table 1) which failed to produce smut in Hannchen produced over 88 per cent of smutted plants in Orel. Tennessee Winter was the only variety in which all collections of spores produced smut.

In the more comprehensive experiment in 1925–26 thirty-two collections of smut were used to inoculate several varieties of barley. Table 3 gives the source and varieties of barley in which each collection of smut was grown since the original collection was made, as well as the smut percentages for Tennessee Winter and Hannchen and in some cases for other varieties inoculated in these experiments.

In no case as shown in Table 3, did a collection of smut which failed to produce smutted plants in Tennessee Winter produce the disease in any other variety of barley inoculated. Spores from many of the collections which produced high percentages of smut in Tennessee Winter failed to produce smutted heads in Hannchen. None of the three smuts used to inoculate Nakano Wase produced smutted plants, but the three smuts used on Alpha, the six smuts used ⌐⌐ Han River, and the seven smuts used on Alaska all produced hiɡ,ı percentages of smutted heads. Two of the three smuts used to inoculate Orel were effective.

As shown in Table 1, spores from Han River barley which had bɔ⌐n inoculated with smut from Tennessee Winter barley produced ⸱00 per cent of smut in Hannchen, whereas spores from Alaska barley

Table 3.—*Effect of inoculating dehulled seed of seven varieties of barley with ...s of loose smut obtained from different sources.* [*Test made in greenhouse at Arlington Farm, Rosslyn, Va., winter of 1925–26*]

Smut lot No.	Source of smut	Varieties from which smut was obtained in—			Percentage of smutted plants in different varieties						
		1923	1924	1925	Tennessee Winter (selection 52)	Hannchen (C.I.531)	Alaska (C.I.4100)	Han River (C.I.200)	Orel (C.I.351)	Alpha (C.I.959)	Nakano Wase (C.I.2166)
Control	Rosslyn, Va.	Tennessee Winter			0	0	0	0	0	0	0
1	do		Alaska	Tennessee Winter	85.7	0					
2	Moorhead, Minn.			Oderbrucker	72.7	0					0
3	Rosslyn, Va.			Nakano Wase	0	72.5				92.1	0
4	do		Han River	Han River	78.6	97.4	42.1	46.2			
5	do	Tennessee Winter	do	do	68.2	97.0	60.0	51.3			
6	do	do	Alaska	Hannchen	93.6	0	59.0	77.1			
7	do	do	Han River	Orel	71.4	100.0	57.5	35.9			
8	do	do	Manchuria	do	81.8	95.0			38.3		
9	Madison, Wis.		Han River	Tennessee Winter	75.8	91.7			67.5		
10	Rosslyn, Va.	Tennessee Winter	Han River	do	75.9						
11	do	do	Han River	Hannchen	0						
12	Madison, Wis.		Wisconsin P. 9	Wisconsin P. 9	0				0		
13	do		Manchuria	Orel	84.0					79.0	0
14	Rosslyn, Va.	Tennessee Winter	Han River	Alpha	0						
15	Ames, Iowa		Han River	Hooded	77.8		71.1			84.6	0
16	Corvallis, Oreg.			Alaska	0		0			0	0
Control	Madison, Wis.		Manchuria	Tennessee Winter	66.7	92.3		0	0		
18	do		Wisconsin P. 6	Wisconsin P. 6	0	0					
19	Rosslyn, Va.	Tennessee Winter	Han River	Tennessee Winter	96.0	0					
20	Egypt.		Unknown	do	98.8	0					
21	Rosslyn, Va.	Tennessee Winter	Alaska	do	93.6	0					
22	do	do	do	do	90.9	0					
23	do	do	do	do	72.4	0					
24	do	do	do	do	74.1	0					
25	do	do	Han River	do	81.3	0					
26	do	do	do	do	86.2	0					
27	do	do	do	do	90.3	0					
28	do	do	do	do	92.9	0					
29	do	do			93.1	2.6					
30	do	do	Alaska		90.6	0	62.5	69.2			
31	do	do	Han River		85.7	5.7	55.0	78.5			
32	Madison, Wis.		Manchuria		2.9	0					
Control					0						

which had been inoculated with the same collection from Tennessee Winter failed to produce smutted plants in Hannchen. The possible occurrence of two strains of smut in the original collection and the influence of the host on the parasite were mentioned as possible explanations of the results of the inoculations. Table 3 shows that seed of Alaska inoculated with smut from Hannchen which came from the Han River variety, the latter in turn having been inoculated from the original Tennessee Winter collection, produced plants which were 60 per cent smutted, whereas in no case did seed of Hannchen inoculated with spores from Alaska or with spores from a smut collection that had been grown in Alaska and then in another variety produce smutted plants. The fact that smut from Hannchen produced smut in Alaska, while the smut in Alaska which came from the same original source as that in Hannchen failed to produce smut in Hannchen, tends to weaken the theory of distinct strains of the fungus specific to Alaska and Hannchen, which the data in Table 1 seem to support. It does seem to strengthen the theory that there is a definite host influence on the fungus which is shown when the smut is transmitted from one variety to another. As previously mentioned, both factors may be concerned in the results. Further study is needed to clear up these points.

A preliminary experiment in 1925 showed that Alpha barley, although apparently infected naturally by loose smut only through the flower, can be infected by inoculating the dehulled seed with loose-smut spores. Spores collected from Alpha in New York in 192-. failed, however, to produce smut either in Alpha or Han River when the dehulled seed was inoculated. The spores might not have been sufficiently viable to produce infection, or it may be possible that a strain of loose smut exists in New York which for some reason infects only through the young embryo at flowering time. On the other hand, there may be morphological factors of the host which prohibit infection in nature except through the flower, even though the de-hulling of the seed would render the seedling very susceptible to infection by seed-borne spores. The three smuts used to inoculate Alpha in the experiment covered by Table 3 produced high percentages of smut in that variety.

Several barley varieties were inoculated from the original collection of loose smut on Manchuria from Madison, Wis., in 1924. In the fall of 1925 two collections of this same smut from Tennessee Winter and one from Orel were used in making the inoculations. One collection from Tennessee Winter produced smut in both Tennessee Winter and Hannchen, although Hannchen inoculated with spores from the original collection failed to become smutted. The other collection produced smut in Tennessee Winter but not in Hannchen. The collection from Orel failed to produce smut in either Tennessee Winter or Hannchen. This may be due to the occurrence of more than one strain of smut in Manchuria or to some other cause. An occasional mutation would explain occurrences of this kind.

Smut from the 1923 collection from Tennessee Winter, which subsequently had been grown in Han River and Alpha, failed to produce smut in Hannchen, but produced high percentages of the disease in Alpha and Tennessee Winter. Perhaps Alpha is smutted by ι. strain which is effective on Alaska but not on Hannchen or a simil.

strain, or it alters the fungus in the same way that Alaska does so that it does not produce smut in Hannchen.

Only one plant of Nakano Wase was smutted in the experiments recorded in Table 1. Previous experiments had given negative results. In 1925, however, a number of heads of loose smut were found in Nakano Wase barley on Arlington Farm. Spores from these smutted heads were used in the experiments recorded in Table 3. No smut was produced in any variety by inoculating the seed. Although the spores were collected and handled with the same care as those of other collections, they might have lost sufficient of their vitality to prevent their causing infection at the time the seed was inoculated and sown. In an experiment conducted by J. W. Taylor and the writers, several heads of Nakano Wase were inoculated by placing the spores in the flower at the time the smutted heads were collected. The seed from the inoculated heads was sown on Arlington Farm the following season. From this seed 26 plants matured, of which 8, or 30.8 per cent, were smutted. As previously mentioned, the spores of some smut strains may remain viable longer than spores of other strains. There may also be strains which infect only through the flower.

CONCLUSIONS

The data obtained in these experiments show that variants exist in the species, *Ustilago nuda*. These variants apparently correspond to what in other fungi are termed strains or physiologic forms. There is some indication, however, that these variants are altered in their pathogenicity by certain hosts. Further study is needed to clear up this point. Various smut collections not only showed differences in their ability to produce smutted heads in barley varieties, but they also affected germination and host development differently. In studying the loose smut of barley it is important that its effects on the host plant throughout its entire development be studied. There may be strains of the loose smut which infect barley in nature only through the floral organs. This might be due either to morphological or physiological characters of the host, to short-lived spores, or to other physiological characters of the fungus. Further experimental study of the subject is needed.

DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND CARYOPSIS OF POA PRATENSIS AND POA COMPRESSA[1]

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INTRODUCTION

As a result of the work of Cieslar, 1883 (*3*),[2] Von Liebenberg, 1884 (*18*), Jönsson, 1893 (*14, p. 40–47*), Hite, 1919 (*11*), Harrington, 1923 (*10*), Toole, 1923 (*29*), and many others, it is known that the seeds of *Poa pratensis* (Kentucky blue grass) require an alternation of temperatures for germination. An exposure of the seeds[3] on moistened blotters at a temperature of about 30° C. for 6 hours, and of 20° for 18 hours each day, results in complete germination of the viable seeds in about 28 days. An exposure of the seeds of *Poa compressa* (Canada blue grass) to a similar daily alternation of temperatures, and to light, and treatment with a dilute nitrate solution, result in complete germination of the viable seeds within three or four weeks.

This investigation was conducted in an effort to discover some of the causes underlying the differences in the germination conditions of the seeds of these two related species. A study of the development of the ovule and especially of the embryo was undertaken to determir ? whether the structure and stage of development of the embryo have any relation to germination conditions. Since polyembryony is frequently observed in seeds of Poa, it was thought desirable to trace the origin of the embryos where more than one is present. A microchemical study of the membranes of the caryopsis showed that during its development many of its structures are so modified that they can no longer be definitely identified in the mature caryopsis. It was necessary, therefore, to make a detailed comparative study of the development of the fruits of these two species, as well as a microchemical study of the membranes of the caryopses, preliminary to a more detailed investigation of the physiology of the germination of these seeds.

No noteworthy differences were found in the development and structure of *Poa pratensis* and *P. compressa;* hence the two species will be described together.

MATERIALS AND METHODS

During the summer periods of 1922, 1923, and 1924 material from plants of *Poa pratensis* and *P. compressa* growing in the vicinity of Washington, D. C., was collected at different stages of development of the panicles.

In January, 1923, and January, 1924, plants of both species were potted and placed in artificially lighted greenhouses at a temperature of 50 to 60° C. Artificial light was used to increase the light period.

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 11
June 1, 1927
Key No. G-572

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The panicles of *Poa pratensis* were in open flower about 2 months after potting. *Poa compressa* flowered 10 days later than *P. pratensis.* Material was collected at frequent intervals before the flowers opened. Each panicle was tagged the day it showed open flowers and a branch or two from a panicle was fixed twice daily at first, and less frequently as the seeds approached maturity. The material was fixed in chromo-acetic, picro-acetic, picro-chromic, Bouin's modified, Carnoy's, acetic alcohol, and Flemming's (weak, medium, and strong) solutions. Xylol was used to precede the paraffin infiltration. Sections were cut 8 to 12 microns thick. Flemming's triple stain, Ehrlich's haematoxylin and safranin, and Haidenhain's haematoxylin were used to stain the slides.

DEVELOPMENT OF THE FEMALE GAMETOPHYTE

Poa pratensis and *Poa compressa* have a simple ovary containing a single ovule. The ovary has two styles. As in other Gramineae the ovule is campylotropous (pl. 1, A). It has two integuments, each of which is composed of two layers of cells except at the base, where there are sometimes three layers (pl. 1, A).

The megaspore mother cell divides and forms two daughter cells which divide again and give rise to a row of four megaspores. Usually the innermost megaspore develops and forms the embryo sac, the other three being digested and absorbed by its growth (pl. 2, A). In several ovules observed, the outermost megaspore develops and forms the embryo sac. Frequently two embryo sacs develop within the same nucellus (pl. 2, C). Details of their development are discussed later. The female gametophyte develops in the usual manner. A two-nucleate gametophyte is shown in Plate 2, B. In the mature gametophyte, the two synergids and the egg nucleus are at the micropylar end, the two polar nuclei lie side by side at the center, and the three very large antipodals lie near the chalaza (pl. 1, B). The egg cell is sometimes spherical and sometimes pear-shaped.

Fertilization was not observed by the writer, although the slides were carefully examined for this detail. The material is not particularly favorable for the study of fertilization, because of its small size. The pollen mother cells have been observed to divide in the usual manner, forming the four microspores which appear perfectly normal. With reference to *Poa pratensis* Nishimura (*23*) says that the pollen grains show no departure and develop and germinate normally.

EXPLANATORY LEGEND FOR PLATE 1

A.—*Poa pratensis.* Longitudinal section of an immature ovary, showing 4 megaspores surrounded by nucellus; inner integument composed of 2 layers of cells; outer integument composed of 2 layers of cells; ovary wall. × 210

B.—*Poa compressa.* Longitudinal section of the female gametophyte surrounded by the nucellus, showing the egg cell and 2 synergids at the micropylar end; 2 polar nuclei at the center; 3 comparatively small antip. ʰls near the chalaza. × 500

C.—*Poa compressa.* Longitudinal section of the embryo sac showing a two-celled embryo at the micropylar end; 2 polar nuclei at the center; 3 very large antipodals near the chalaza. × 500

D.—*Poa compressa.* Longitudinal section of the embryo sac, showing a three-celled embryo at the micropylar end; 2 polar nuclei fused to form the endosperm nucleus at the center; 3 large antipodals near the chalaza. × 500

KEY TO ABBREVIATIONS USED IN PLATES AND FIGURES

a, antipodals; *pn*, polar nuclei; *en*, primary endosperm nucleus; *e*, egg; *syn*, synergids; *em*, embryo (when more than one embryo sac is present the parts of the embryo sac more distant from the micropyle are designated by *a′*, *pn′*, *en′*, *e′*, *syn′*, *em′*); *sc*, scutellum; *vs*, vascular strand; *cl*, coleoptile; *fl*, foliage leaves; *v*, vegetative cone; *ep*, epiblast; *r*, radicle; *rc*, root cap; *co*, coleorhiza; *ii*, inner integument; *oi*, outer integument, *nu*, nucellus; *ch l*, chlorophyll layer; *ow*, ovary wall; *s*, suberin; *c*, cutin; *ec*, epidermal cells.

In some of the photomicrographs the cell walls and the outlines of the nuclei have been traced in ink.
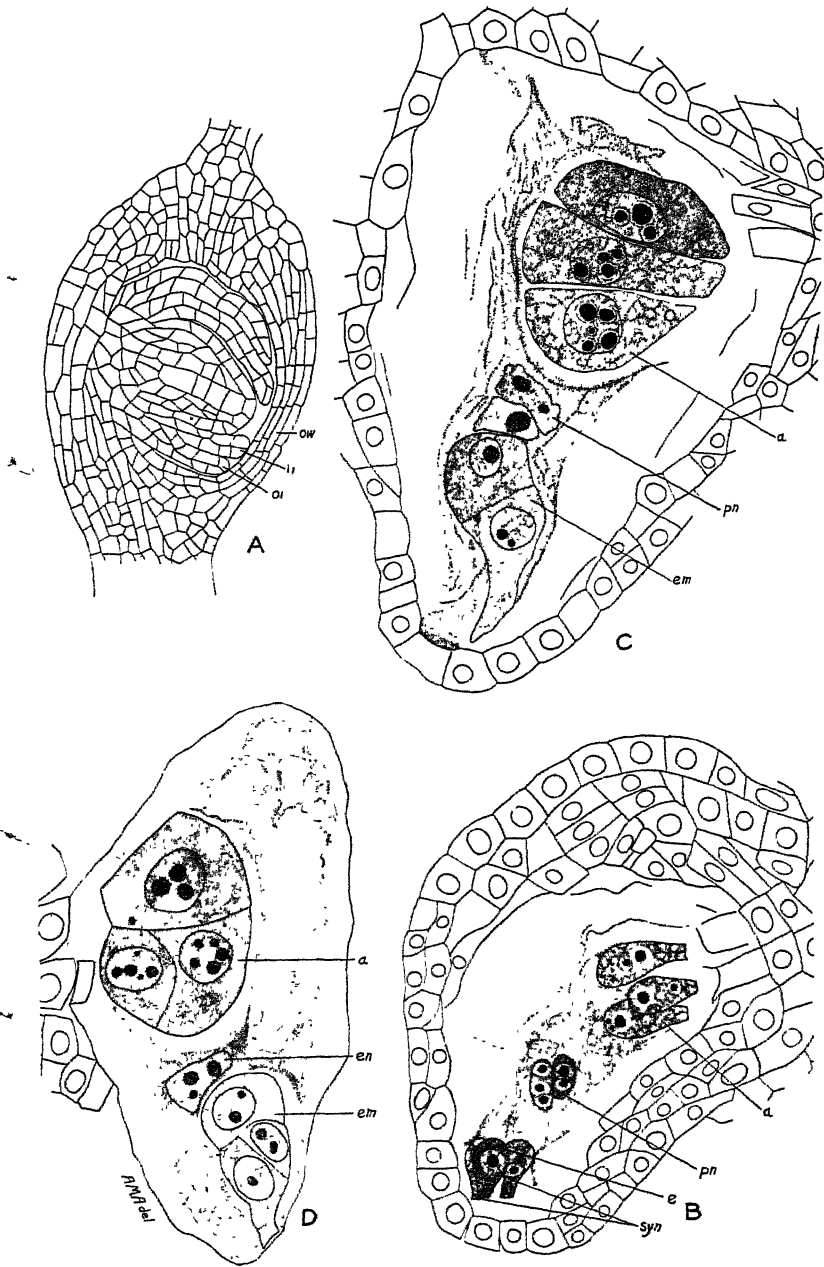
(For explanatory legend, see p. 1002)
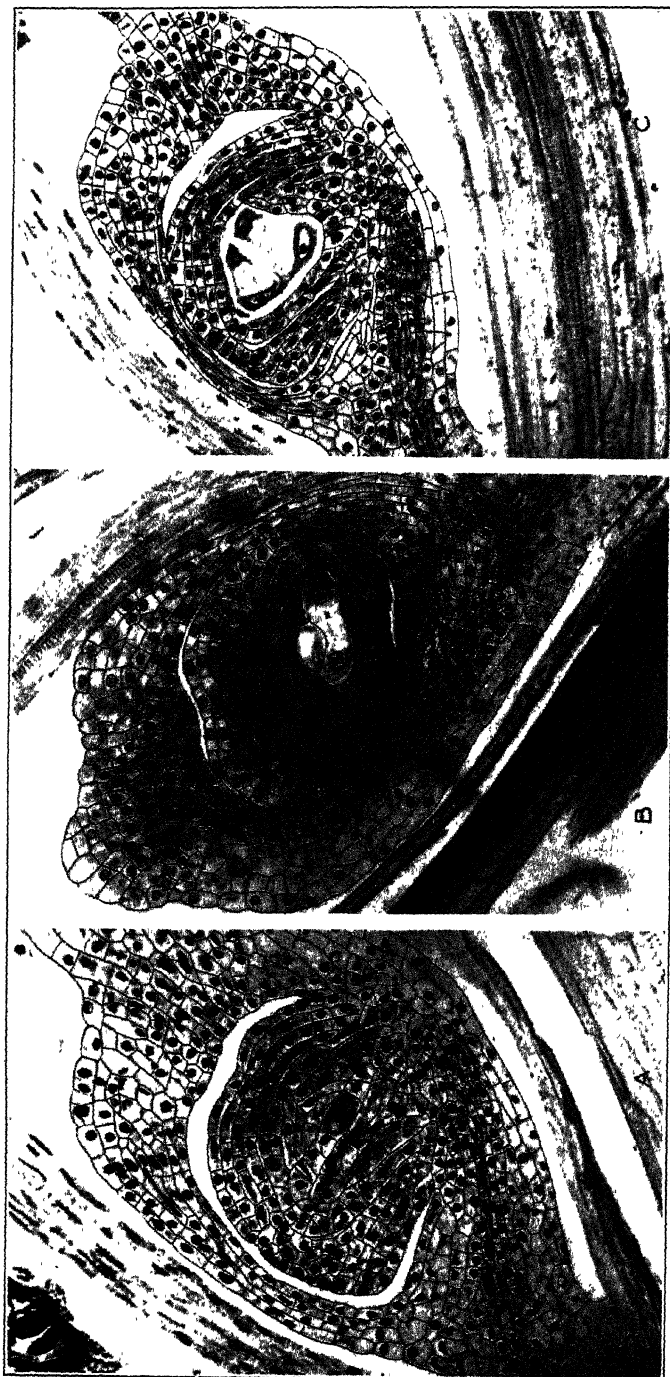
A.—*Poa pratensis.* Photomicrograph of a longitudinal section of a young ovary, showing four megaspores. The deepest megaspore is developing to form the embryo sac. × 250

B.—*Poa pratensis.* Photomicrograph of a longitudinal section of a young ovary, showing two embryo sacs lying side by side within the same nucellus. One embryo sac is two-nucleate, the other is one-nucleate, but its nucleus is not visible in this section. × 200

C.—*Poa pratensis.* Photomicrograph of a longitudinal section of a young ovary, showing two embryo sacs within the same nucellus. Each embryo sac is in the two-nucleate stage. × 200

ANTIPODALS

The marked development of the antipodal cells is a notable feature in *Poa compressa* and *P. pratensis;* in fact, the Gramineae are conspicuous for their strongly developed antipodals. Johannsen, working on Hordeum (*15*), Koernicke on Triticum (*17*), and Cannon on *Avena fatua* (*1*), found that the antipodals increase in number to 36 or more before fertilization and begin to disorganize with the beginning of the endosperm development.

With the growth of the embryo sac, the antipodals of *Poa compressa* and *P. pratensis* increase greatly in size in comparison to the egg apparatus. Their position at the chalaza, which is supplied with nutriment through the vascular strand, insures a plentiful supply of food for their growth (pl. 1, C). Each antipodal contains a very large nucleus with several nucleoli. The antipodals are densely filled with protoplasm and take a very deep stain. They contain a few vacuoles even in the earlier stages (pl. 3, A, pl. 4. B), but more as they disintegrate. Three very large antipodals are generally associated with one embryo (pl. 1, B, C, D). However, four and five antipodals are found very frequently (pl. 3, A, and pl. 5, A). Although the antipodals of Poa do not increase in number to the same extent as the antipodals described by Johannsen in Hordeum, Koernicke in Triticum, and Cannon in *Avena fatua,* they are perhaps more conspicuous by their great size and dense protoplasm.

When two embryo sacs are found in the same nucellus, six or more antipodals are always present. In Plate 3, B, the three very large antipodals are associated with the embryo at the micropyle, and the other five antipodals overlying these three are associated with the second embryo more distant from the micropyle. Although the seeds showing polyembryony might at first glance be interpreted as containing a great increase in number of antipodals, close observation shows that they conform to two or more sets of three or more antipodals for each embryo sac (pl. 4, B). In one case (pl. 6, C), the antipodals (associated with the micropylar embryo and the endosperm filling one-half of the seed) are practically disintegrated, while the four antipodals (associated with an embryo not visible in the photomicrograph, and an undivided endosperm nucleus) persist very prominently.

The antipodals persist until late in the endosperm formation, when they become vacuolated and disintegrate. They are finally crowded off to one side and are presumably digested and absorbed by the growing endosperm.

DEVELOPMENT OF THE ENDOSPERM

By a series of rapid divisions the primary endosperm nucleus forms many free nuclei in a parietal layer of cytoplasm and in a mass about the embryo (pl. 6, A). A parietal layer of cells soon forms. The cells of the parietal layer divide, forming quite definitely two rows of cells except toward the embryo, where the cells divide more rapidly. Each cell of these two rows then divides again, filling in toward the central cavity. Gordon (*8*) found that in the formation of the endosperm of wheat, barley, and oats "the lining layer of the embryo sac assumes the character of a cambium, which

ᵣ oduces segment cells only on its inner surface." In *Poa pratensis*
and *P. compressa* the cells of the inner row are equally as active in
division as the cells of the outer row. Practically every cell of the
inner row has been observed in a stage of division.

When endosperm formation is practically complete, the cells of
the outermost layer of the endosperm divide, and the outer row of
cells arising from this division differentiates to form the aleurone
layer. The aleurone layer is composed of only one layer of cells,
but occasionally two cells are superimposed one above the other.
The aleurone cells surround the endosperm and embryo except at
the base of the embryo.

There are several rows of nucellar cells present which are about
the same size as the aleurone cells when the aleurone cells are first
differentiated. The outermost nucellar cells are the only ones that
form a definite and distinct row. At maturity two or more rows of
nucellar cells remain and are compressed like the cells of the inner
integument.

DEVELOPMENT OF MORE THAN ONE EMBRYO SAC WITHIN THE SAME NUCELLUS

Polyembryony occurs frequently in *Poa pratensis* and *P. compressa*. This was also observed by Zinn (*33*) in the germination of
both of these species and by Nishimura (*23*) in the germination of
P. pratensis. A review of the literature on polyembryony is given
by Ernst (*6*). Polyembryony has been described as arising in various
ways by many authors.

There is much well-illustrated literature on polyembryony in
which embryos are described as arising from the nucellus, suspensor,
and synergids. The presence of more than one embryo sac within
the same nucellus is briefly mentioned by various authors, but
except in rare instances only one embryo sac is thought to de-
velop an embryo. In *Rosa lirida*, Strasburger (*28*) noted more
than one embryo sac within the ovule, but found that only one
developed. Schwere (*26*) states that in *Taraxacum officinale* in
one case he found within one ovary two well-developed embryo
sacs, each containing a normal healthy embryo with the coty-
ledons differentiated. Schacht (*25*) found that in *Cherianthus
cheiri* several embryo sacs are developed, only one of which, the
largest, becomes fertilized. Hofmeister (*12*) observed in Rosa sp.
three to six embryo sacs only two of which usually contained egg
cells. Jönsson (*13*) states that in *Trifolium pratense* more than one
megaspore develops, and that more than one embryo sac within
the same nucellus contains an egg which is fertilized. Mottier (*22*)
observed in Delphinium two mature embryo sacs lying side by side
in one ovule, but did not determine whether more than one embryo

Plate 3

Development of Poa pratensis and P. compressa

A

B

was fertilized and whether more than one embryo reached maturity in a single seed. In the genus Smilacina McAllister (*19*) occasionally found within the same nucellus two embryo sacs which arose from two megaspore mother cells. These megaspore mother cells are usually separated more or less by somatic cells. Martin (*20*) found that in *Medicago sativa* the number of archesporial cells ranged from one to six and that more than one usually occurs. Two to four rows of megaspores may occur in the same nucellus and often more than one megaspore starts to form an embryo sac, but not more than one embryo sac matures.

The results of the present study seem to indicate that polyembryony in *Poa pratensis* and *P. compressa* results from the development of more than one embryo sac within the same nucellus. With the development of the megaspores, the young embryo sacs are crowded from their original positions in such a manner that it is difficult to determine whether they originate from two separate megaspore mother cells or from two megaspores of a single row of megaspores (pl. 2, C). In one ovule two parallel rows of four megaspores each were observed. In another ovule two 1-nucleate embryo sacs were found lying side by side. In another ovule (pl. 2, B) a 1-nucleate and a 2-nucleate embryo sac were present side by side. However, in two other ovules two 1-nucleate embryo sacs were observed one above the other, apparently in the same row. The two megaspores develop in the usual manner. Each of the well-developed embryo sacs contains an egg cell and two synergids at the micropylar end, the two polar nuclei at the center of the embryo sac, and three or more large antipodals near the chalaza. With the growth of the ovule the two embryo sacs usually lie side by side diagonally across the ovule from micropyle to chalaza. In a later stage of the development of the embryo sac, the one embryo often lies at the micropyle and the other embryo slightly above it, as shown in Plate 3, B, and Plate 4, A. The position of the embryo sacs relative to the chalaza seems to be an important factor in their development. If the embryo sacs are situated equally near to the chalaza and vascular strand, they develop simultaneously. The embryo sac (usually the one at the micropyle), which is situated nearer to the source of food, develops more rapidly. This condition is shown in Plate 7, A, where the embryo sac farther from the micropyle, which is in the 8-nucleate stage, is pushed up above the chalaza and the vascular strand, while the embryo sac at the micropyle is situated nearer the vascular strand and has developed an embryo of three or more cells. In many of the seeds both embryos are at about the same stage of development and each set of antipodals is in close contact with the chalaza (pl. 3, B, and pl. 4, A).

In the early stages a developing endosperm·is associated with each embryo, although both endosperms may not continue to develop (pl. 6, B). At this stage of development, as shown in Plate 6, B, one seed was found containing three embryos each associated with a peripheral layer of endosperm. At a slightly later stage in the development of the embryos several seeds showed the two embryos about equally developed, the endosperm of the embryo at the micropyle filling one-half of the nucellar cavity, while the endosperm nucleus of the embryo more distant from the micropyle had not divided (pl. 7, B, and pl. 6, C). In these seeds the endosperm nucleus of the embryo sac farther from the micropyle does not appear to have much chance of developing further, as the antipodals (from which the endosperm is probably supplied with food) are pushed away from the vascular strand. A later stage was observed in which the endosperm associated with the embryo at the micropyle filled three-fourths of the nucellar cavity, and the endosperm nucleus associated with the embryo farther from the micropyle was pushed to the distal end of the seed, away from the embryo that was previously connected with it.

In the sections of all the mature seeds in which more than one embryo was observed, each embryo was found to be complete in itself (pl. 8, C). The two mature embryos are in the same relative position as the younger embryos, both near the micropyle, one slightly above the other. Only one endosperm surrounded by the aleurone layer is generally observed in the mature seed. Some seeds containing two embryos were observed, but with one endosperm and the aleurone layer almost filling the nucellar cavity. However, a smaller endosperm was present which in one instance occupied a small space toward one side and in other instances was almost completely surrounded by the larger endosperm.

Seeds were observed in which in one case a two-nucleate, in another case a four-nucleate, and in still another case an eight-nucleate embryo sac was surrounded by the antipodals of a further developed embryo sac. A four-nucleate embryo sac surrounded by the antipodals associated with the embryo at the micropyle, is shown in Plate 5, B. Such embryo sacs probably do not develop two embryos.

At maturity the two embryos are not always equally developed. Sometimes one embryo is mature while the other embryo is only about one-half grown (pl. 8, B).

Nishimura (*23*) states that in *Poa pratensis* several embryos may arise from a massive suspensor and embryonic buds may also develop from the antipodal nucellar region, and that embryos appear less commonly from the nucellar tissue in other regions of the embryo sac. The writer has observed in sections of several grains the seed entirely filled with endosperm surrounded by the aleurone layer, with one mature embryo at the micropyle and with another embryo about

Plate 6
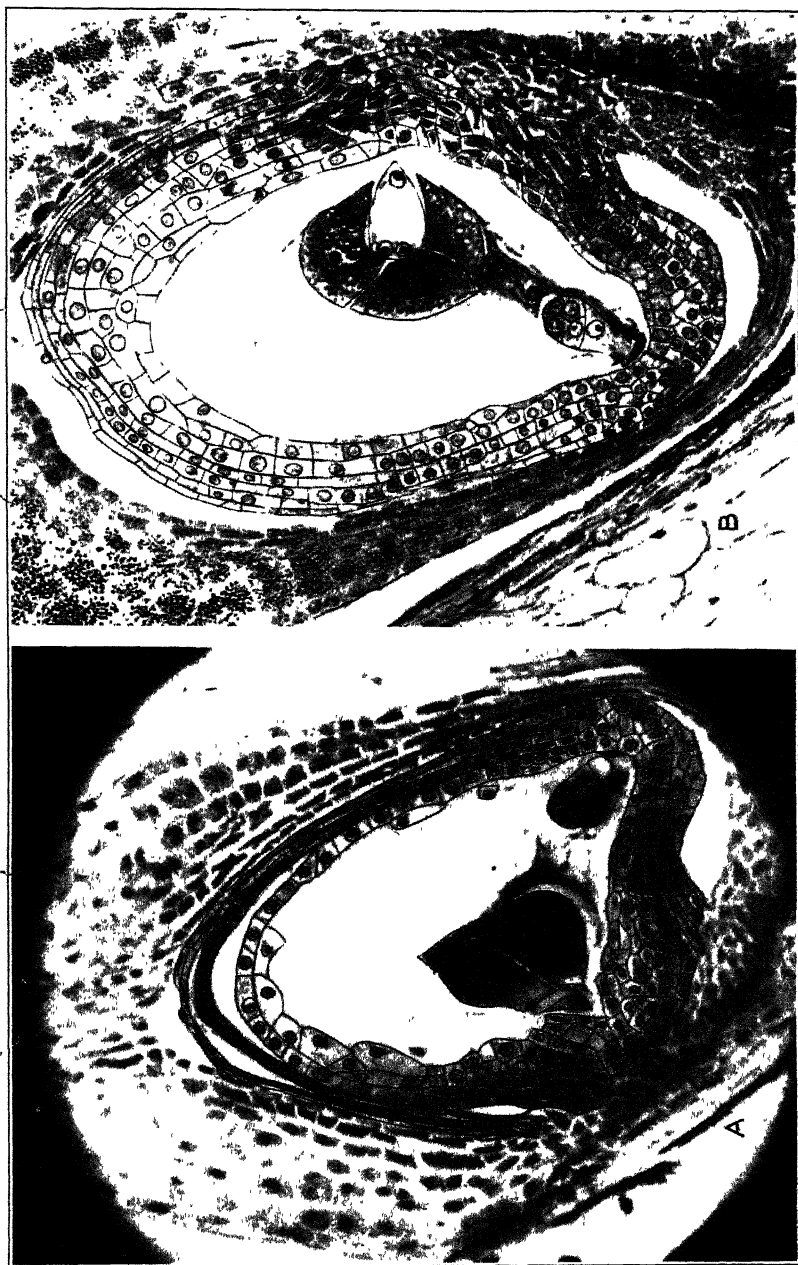
one-half grown outside the aleurone layer. The writer has never found any indication that this is a nucellar embryo. In contrast to Nishimura's interpretation, it is assumed from observations of earlier stages (pl. 3, B, and pl. 7, B) that the embryo outside the aleurone layer is an embryo from a second embryo sac which has been pushed to the side by the growing embryo and endosperm situated more closely to the vascular system.

In germinated seeds showing more than one embryo, each embryo is complete with its own scutellum, plumule, and root. No embryos were observed showing two plumules associated with one radicle or two radicles associated with one plumule.

DEVELOPMENT OF THE EMBRYO

The homologies of the scutellum, epiblast, and plumule sheath of the grass embryo have been much discussed. Kennedy (*16*) gives an extensive review of the work done by earlier authors such as Sachs, Bruns, Hofmeister, Richard, Adr. de Jussieu, Malphigi, Mirbel, Van Tieghem, and many others. Worsdell (*32*) and Coulter (*5*) have written more recently on the subject. The homologies of the parts of the embryos of *Poa compressa* and *Poa pratensis* will not be discussed here; only the development of the embryos will be traced.

Before any external differentiations can be detected the embryo is composed of a number of cells (fig. 1, A, B, C). A slight indentation of the front face is the first external differentiation which indicates the development of growing points (fig. 1, D). With further growth of the embryo, one growing point above the original indentation and two growing points below the original indentation are observed. The growing point directly below the first indentation develops to form the vegetative cone. The growing point above the first indentation and the growing point below the vegetative cone are rudiments of tissue which, with further growth of the embryo, unite to form the plumule sheath or coleoptile encircling the plumule (fig. 1, E).

One section of a slightly older embryo shows these two rudiments of the plumule sheath to have grown until they met, thus forming a complete ring of tissue about the plumule (fig. 1, G). In the adjacent section of the same embryo the two rudiments of the plumule sheath are not united (fig. 1, H). This incomplete union of the plumule sheath may account for the small slit or opening described by other writers in the mature embryos of grasses. One section without the other at this stage could very easily lead one to entirely different interpretations of the formation of the plumule sheath or coleoptile. Hanstein (*9, pp. 47–80*) describes the plumule sheath as formed by the meeting of two outgrowths in Brachypodium; Souèges (*27*) figures the plumule sheath as formed similarly in *Poa annua*.

EXPLANATORY LEGEND FOR PLATE 6

A.—*Poa pratensis.* Photomicrograph of a longitudinal section of an immature ovary, showing endosperm formation in which free nuclei lie in a peripheral, cytoplasmic layer. A one-celled embryo is present. ✕ 100

B.—*Poa pratensis.* Photomicrograph of a longitudinal section of an immature ovary, showing two embryos, each with its own endosperm. ✕ 100

C.—*Poa pratensis.* Photomicrograph of a longitudinal section of an immature ovule, showing one embryo with its endosperm filling one-half of the nucellar cavity. The antipodals have apparently been absorbed. Another embryo which is not visible in this section slightly above the first embryo is associated with the cytoplasm present in the upper part of nucellar cavity and four antipodals which are near the chalaza. ✕ 100

Fig. 1.—(For explanatory legend, see p. 1009.)

The epiblast is differentiated at about the time the plumule sheath is formed. The radicle has not been formed at this stage (fig. 1 F).

The mature embryo (fig. 2 and pl. 8, A) in the ripe seed in both species of Poa is situated near the micropyle and lies against the side opposite the chalaza. The scutellum, which is considered the organ of absorption through which the growing parts of the embryo receive their nutriment from the endosperm during germination, is shieldlike in appearance. Near the base of the plumule it is composed of only a few layers of cells but is thicker toward the top. The epidermis, or the layer of cells of the scutellum in contact with the endosperm, does not form a palisade layer as described for some grasses. The plumule has developed into a vegetative cone with two or more rudimentary foliage leaves. Except for a small opening it is surrounded by the coleoptile, or plumule sheath. The primary root is not conspicuously well developed and terminates with a root cap. The radicle and root cap are surrounded by the parenchymatous tissue of the coleorhiza. The primordia of

FIG. 2.—*Poa pratensis.* A composite drawing of successive longitudinal sections of a mature embryo, showing scutellum with a poorly defined epithelial layer and with a procambium strand; vegetative cone with the first and second leaves; coleoptile surrounding the plumule; primary radicle terminating with a root cap; the coleorhiza surrounding the radicle and root cap; epiblast. × 280

EXPLANATORY LEGEND FOR FIGURE 1

A.—*Poa compressa.* Longitudinal section of a young embryo composed of 3 cells. × 310
B.—*Poa compressa.* Longitudinal section of a young embryo composed of 9 or more cells. × 310
C.—*Poa pratensis.* Longitudinal section of a young embryo composed of 14 or more cells. × 310
D.—*Poa pratensis.* Longitudinal section of a young embryo, showing indentation which indicates growing points above and below it. × 310
E.—*Poa compressa.* Longitudinal section of an immature embryo. The projection below the first indentation is the vegetative cone. The projection below the vegetative cone and the one above the first indentation are rudiments of the coleoptile. A smaller embryo lies over the lower left half of this embryo. × 310
F.—*Poa compressa.* Longitudinal section of an immature embryo. The coleoptile appears completely to surround the vegetative cone. The epiblast is beginning to be differentiated. × 310
G, H.—*Poa compressa.* Two consecutive longitudinal sections of the same immature embryo. In one section (G), the coleoptile appears completely to surround the plumule. In the following section (H), the lips of the coleoptile are not united. A rudimentary epiblast is present. ♣ 310
For key to abbreviations in figures see page 1002.

secondary roots are not present. A procambium strand leads from the tissue between the plumule and radicle into the upper part of the scutellum. A well-defined epiblast is developed. The epiblast has no vascular strand.

Poa compressa and *P. pratensis* have a complete embryo at maturity. No differences were found in the development of the embryo of these two species which would account for the differences in the physiology of their germination.

INSECT EGGS ON OVARIES OF POA COMPRESSA

When the glumes from the immature pistils of *Poa compressa* were dissected preparatory to a microchemical study of the developing ovule and ovary, a small protuberance was observed on some of the ovaries. It occurs most frequently near the base of the style, although it is. found sometimes on the side of the ovary. The ovaries thus affected are lighter in color, somewhat inflated, and slightly larger than the normal green ones. About 20 per cent of the pistils dissected for microchemical studies from material collected at Yonkers, N. Y., showed this minute swelling. Ovaries with protuberances were fixed in Flemming's medium solution, embedded in paraffin, sectioned 12 microns, and stained with Flemming's triple stain.

Examination of the sections showed that these protuberances were not plant tissue but had the appearance of insect eggs. In some cases, one end of the egg extends to a depth of two or three cells in the ovary wall. In one instance, it is partially embedded in the loose tissue beneath the style. In another instance, about one-half of the egg is embedded deep in the tissue of the ovary wall and is in close contact with the outer integument of the ovule.

These slides were examined by G. F. White, of the Bureau of Entomology, United States Department of Agriculture, who observed that the "protuberances" are insect eggs in various stages of development, from a quite early stage to a late one, in which the insect is nearly ready to emerge (pl. 9, A, B, C, D).

From a study of the sections, the shape of the eggs appear to be in general reniform. They range from 84 to 100 microns in width. and from 134 to 185 microns in length.

W. A. Riley, head of the department of animal biology of the University of Minnesota, to whom the slides were submitted for further advice regarding classification of the insect, states:

The insect embryos on the young ovaries of Canada bluegrass which you sent me are those of one of the Thysanophera or 'thrips.' These insects are very common in various flower heads. As far back as 1875 Professor Comstock noted. that they were doing great damage to timothy and June grass in New York State. Since that time the species *Anaphothrips obscurus* has been reported from a wide series of grasses, especially in the Northeastern States. It is quite probable that your specimens belong to this same species.

EXPLANATORY LEGEND FOR PLATE 7

A.—*Poa pratensis.* A composite drawing from successive longitudinal sections of an immature ovary, showing 2 embryo sacs within the same nucellus. The embryo sac at the micropyle contains an embryo of 3 or more cells, endosperm nucleus at the center, and 3 large antipodals near the chalaza. The embryo sac farther from the micropyle contains an egg cell and 2 synergids, 2 polar nuclei not fused, and 3 antipodals near the chalaza. × 415

B.—*Poa pratensis.* A composite drawing from successive longitudinal sections of an immature ovary, showing polyembryony. The embryo at the micropyle is associated with the endosperm, filling one-half of the seed. The embryo farther from the micropyle is associated with the undivided endosperm nucleus and cytoplasm pushed toward the distal end of the nucellar cavity. The remains of antipodals are present. × 143

For key to abbreviations, see p. 1002.
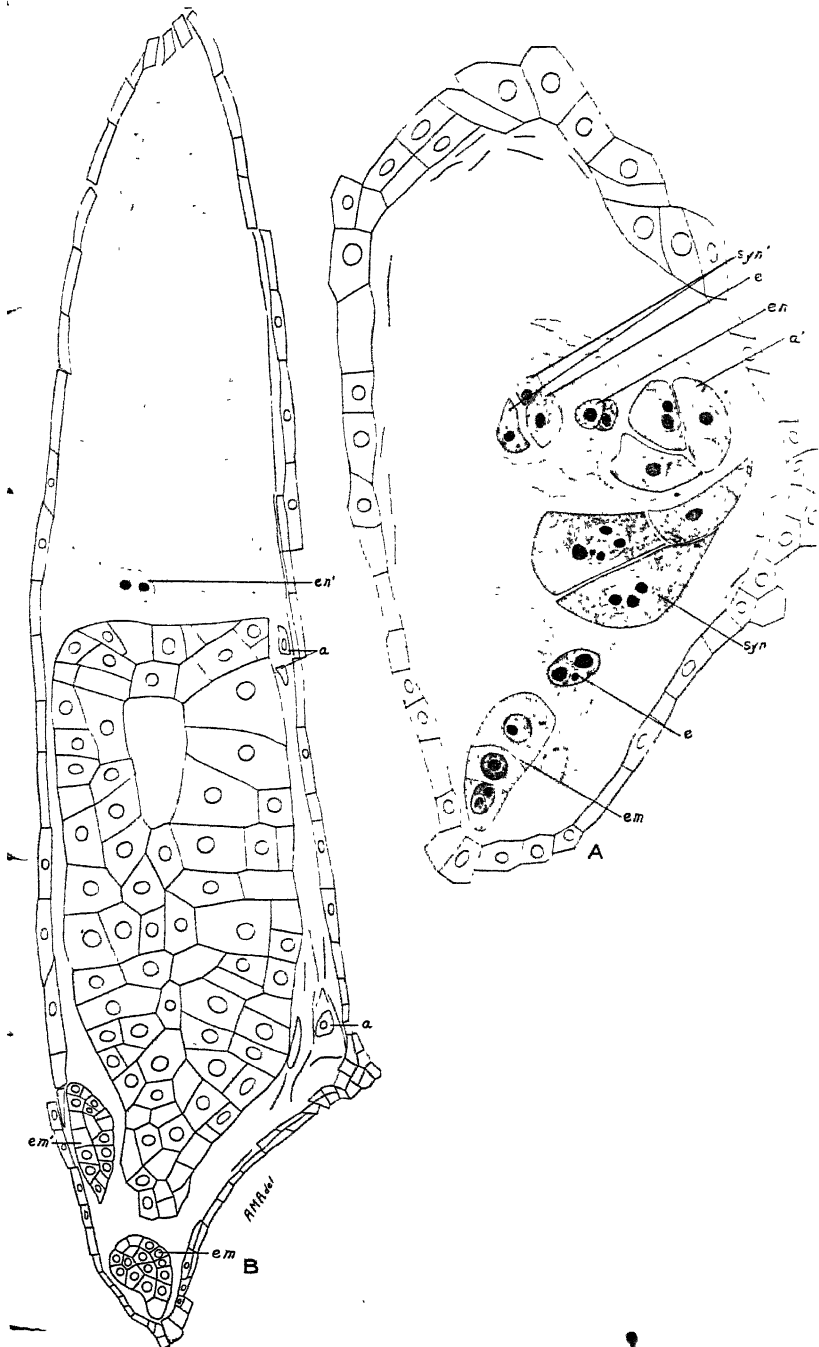
(For explanatory legend see p. 1040)
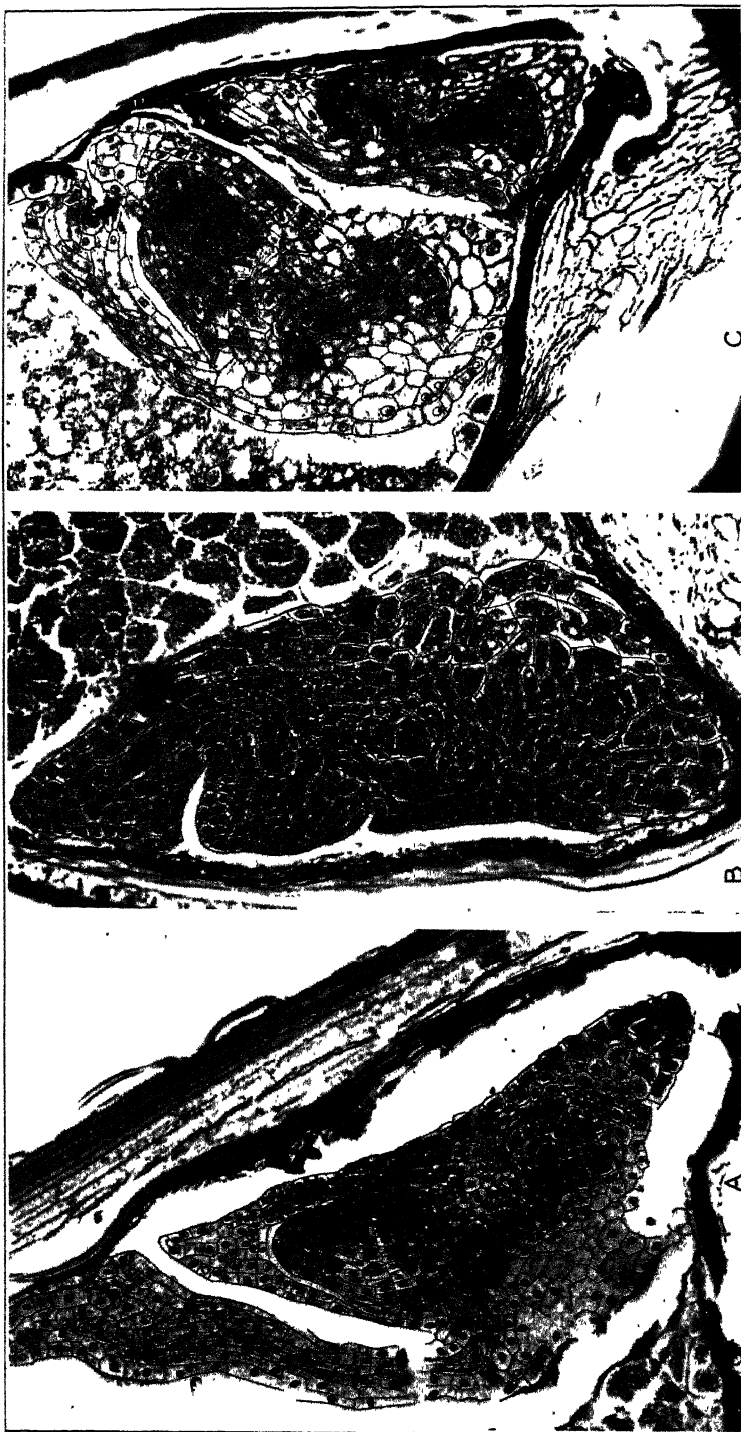
Osborn (*24, p. 155*), Comstock (*4, p. 127*), Fernald and Hinds (*7*), Cary (*2*), and others have described the larva, pupa, and adult stages of *Anaphothrips obscurus* Mull. (*A. striatus* Osb.) and its feeding habits. The insect has an incomplete metamorphosis. Fernald and Hinds found that the eggs are deposited in the tissues of the fresh and tender parts of the leaf. The young feed on the head just as it is making its appearance, and upon the juices from the young tender stem inside the sheath. The young insects which enter the top sheath and suck the stem dry above the upper node cause "silver-top." The adults feed upon the leaves and external parts of the grass, and suck the sap from the cells. The empty cells then appear shrunken and white. Fernald and Hinds estimate that in Massachusetts about 40 per cent of the heads of *Poa compressa* are injured with "silver-top." They cite many other hosts. Cary found that in the early months *Anathothrips striatus* confines itself chiefly to June grass, *P. pratensis*, and that as the season advances it is found quite abundantly in timothy, *Phleum pratense*, and on several species of Panicum, Agrostis, and Festuca.

Nishimura (*23*) states that occasionally slightly larger pistils than normal ones were found. He says:

These were characterized by protuberances. This type of pistil may be due to the deposit of eggs by an insect * * *. Apparently polyembryony and other irregularities are associated with abnormal ovaries. The abnormality arises at different periods in the development of the ovule. In some cases it is associated with the growth of the megaspore, which is seen to become greatly enlarged. Its nucleus at first increases decidedly in size, and this is followed by a massing of the chromatic material, and then its final dissolution. Such megaspores never divide and finally appear as empty sac, often quite equaling in size that of the normal embryo sac * * *. These manifestations of abnormality appear to be associated with the sting of insects which deposit their eggs in the ovary.

In her study of polyembryony the writer has not found an insect egg on an ovary of *Poa compressa* or *P. pratensis* containing two embryo sacs which with further development would have given rise to two embryos. She does not agree with Nishimura, therefore, that insect eggs are associated with polyembryony. Furthermore, from a study of the material at hand the writer is inclined to the belief that some of the ovaries on which insect eggs are deposited do not develop.

DEVELOPMENT AND MICROCHEMISTRY OF THE MEMBRANES OF THE CARYOPSIS [4]

The development of the caryopsis of *Zea mays*, of *Triticum vulgare*, and of *Avena sativa* has been studied by True (*30*). The writer has found no literature referring to the smaller caryopses.

EXPLANATORY LEGEND FOR PLATE 8

A.—*Poa pratensis*. Photomicrograph of a longitudinal section of a mature embryo, showing scutellum, fibrovascular strand, vegetative cone, coleoptile, radicle, root cap, and coleorhiza. The epiblast is differentiated but not distinguishable in photograph. × 200

B.—*Poa pratensis*. Photomicrograph of a longitudinal section showing polyembryony. One embryo is completely developed. The other is only partially developed and crowded directly beneath it. × 210

C.—*Poa pratensis*. Photomicrograph of a longitudinal section of a ripened seed, showing polyembryony. The two embryos are almost equally developed. × 180

The material used for the study of the development of the cary-opses of *Poa pratensis* and *P. compressa* was collected in the vicinity of Madison, Wis., in the summer of 1923, and at Yonkers, N. Y., in the summer of 1924. Living plants with the panicles in the different stages of development were brought into the laboratory and placed in water. The ovaries were sectioned with a freezing microtome or placed directly in paraffin and sectioned with a rotary microtome. Microchemical tests of the membranes were then made on these sections. The methods used in the microchemical work were those suggested by Eckerson,[5] Tunmann *(31)*, and Molisch *(21)*.

Both species of Poa have a simple ovary containing a single ovule (pl. 1, A). The ovary wall is of irregular thickness, being thicker at the top. It consists of an outer epidermis and four or five rows of paren-chymatous cells containing much starch. The inner row of cells of the ovary wall consists of chlorophyll-bearing cells, which give the characteristic green color to the young ovary. When the ovule has reached the four-megaspore stage, it is campylotropous. The ovule is attached to the side of the ovary with the micropyle toward the bottom. The integuments do not completely surround the nucellus at this stage. Later the inner integument surrounds the nucellus completely. Each integument is composed of two layers of cells, except that at the base there are sometimes three layers of cells. Both integuments contain starch. At about the time of fertilization the cell walls of the inner integument, adjacent to the nucellus become slightly suberized (fig. 3, A).

At the stage when the embryo is very small and undifferentiated, little or no endosperm has been formed and the antipodals are very large (fig. 3, B), the cell walls of the ovary wall, of the inner and outer integuments, and of the nucellus contain pectic substances. The cell walls of the inner integument adjacent to the nucellus and the cell walls of the outer integument adjacent to the inner integument are suberized. Usually the suberized layer of the inner integument develops earlier than the suberized layer of the outer integument. The walls of the inner cells of the lemma and palea contain cellulose. No lignin is found at this time.

A little later (fig. 3, C, D), when the aleurone cells are differentiated, but still have thin walls, and the caryopsis is still green, the cell walls of the ovary wall and of the aleurone layer contain pectic substances. The cell walls of the ovary wall, and of the lemma and palea give a cellulose reaction. The suberized layer of the cell walls of the inner integument adjacent to the nucellus has thickened somewhat but not as much as the suberized layer of the inner cell walls of the outer integument adjacent to the inner integument. The protoplasm and unsuberized cell walls of the outer integument begin to collapse at about this stage. They are presumably being dissolved and will leave only the thick layer of suberin. In the earlier stages several cases have been observed where all the cell walls of the outer integument were slightly suberized. It may be that these cell walls also persist as thin suberized layers uniting to form the heavy band of suberin. The nucellus, except for one or two cell layers, has been digested and absorbed at this stage.

[5] Mimeographed outlines of Methods of Microchemistry.

Photomicrographs of longitudinal sections through young ovaries of *Poa compressa* and the eggs of
Anaphothrips obscurus, in different stages of development

A.—Egg in early stage, containing much yolk material. × 100
B.—Egg, showing much yolk material and a developing embryo at one end. × 100
C.—Egg in a later stage of development than B. × 100
D.—Egg, showing insect almost ready to emerge. × 100
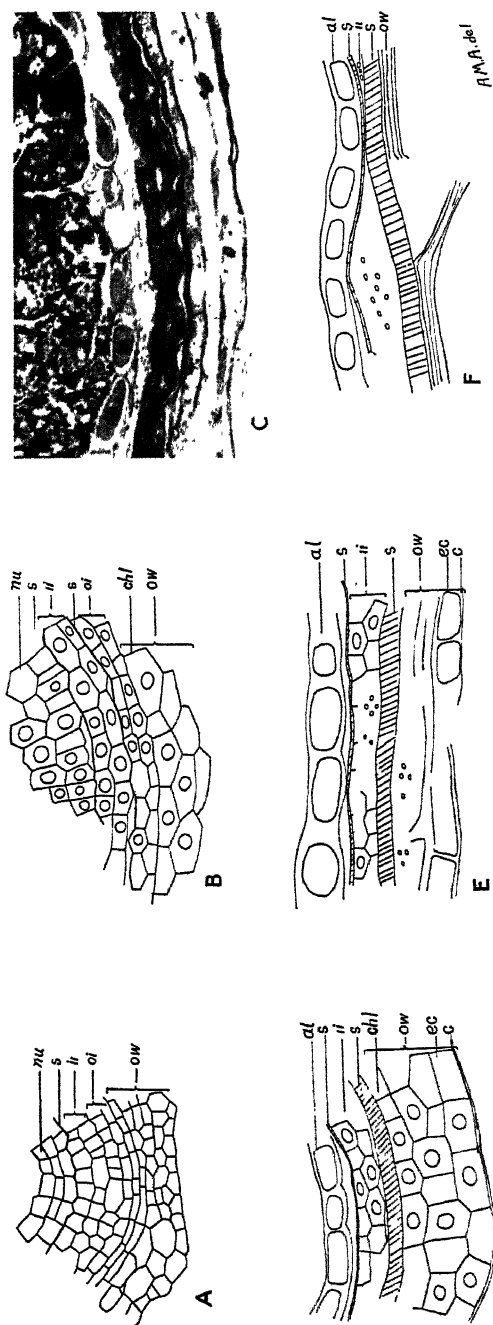
FIGURE 3

A.—*Poa pratensis.* A portion of a longitudinal section of a very young ovule, showing nucellus; inner integument composed of 2 rows of cells and its inner wall slightly suberized; outer integument composed of 2 rows of cells; ovary wall of about 5 rows of cells, of which the inner layer is the chlorophyll layer. × 310

B.—*Poa pratensis.* A portion of a longitudinal section of a young ovule, showing nucellus; 2 integuments, of which the inner layer of the inner and of the outer integuments are slightly suberized; and a part of the cells of the ovary wall. × 310

C.—*Poa compressa.* Photomicrograph of a portion of a longitudinal section of an immature ovule, showing aleurone cells with thin walls; 1 or 2 rows of nucellar cells; inner walls of inner integument suberized; a layer of suberin as the only remnant of the outer integument; ovary wall. × 310

D.—*Poa compressa.* A portion of a longitudinal section of a slightly older ovule than C, showing aleurone cells with thin walls; inner walls of inner integument suberized; a layer of suberin as the only remnant of the outer integument; ovary wall with the epidermis slightly cutinized. × 310

E.—*Poa compressa.* A portion of a longitudinal section of an ovule almost mature, showing aleurone cells with thick cell walls; inner integument with its inner walls suberized, cells less turgid, somewhat shrunken and collapsed, containing some starch; ovary wall cells beginning to collapse. × 310

F.—*Poa compressa.* A portion of a longitudinal section of a mature ovule, showing aleurone cells with thick walls; cells of inner integument compressed, and inner layer suberized; outer integument only a thick layer of suberin; ovary wall cells collapsed and compressed into a thin layer. ×·310

For key to abbreviations, see page 1002. Drawings made from sections of fresh material.

At a later stage, (fig. 3, E) when the caryopsis is fully grown, but green, and when the cell walls of the aleurone cells have become thicker, both they and the cell walls of the ovary wall contain pectic substances and cellulose. At this stage the suberized layer of the outer integument has become a very thick layer. This heavy suberized layer is all that now remains of the outer integument. The inner cell walls of the inner integument are suberized as in the previous stage. Their cells are not dissolved and do not disappear but they are somewhat collapsed. The cell walls of the ovary wall also are beginning to show signs of collapse. The walls of the inner cells of the lemma and palea give a cellulose reaction. The rachilla and the walls of the outer cells of the lemma and palea give a lignin reaction.

When the caryopsis is fully grown and mature (fig. 3, F), the cell walls of the aleurone layer, ovary wall, and lemma and palea give a cellulose reaction and also contain pectic substances. The cell walls of the inner integument adjacent to the nucellus form a comparatively thin layer of suberin. The two rows of cells of the inner integument have collapsed somewhat, forming a narrow layer against the suberized layer of the outer integument. A layer of suberin is all that remains of the outer integument. The suberized layers of the inner and outer integuments are so tightly compressed that their identity can be seen only where the seed coat is torn in sectioning. They then appear as two distinct layers, the outer being much thicker than the inner. The pericarp with its characteristic inner chlorophyll layer has also collapsed and forms a thin layer of about the same thickness as the suberin layer. The chlorophyll layer is no longer green, but brown. It gives to the caryopsis a brown color just before maturity. A few starch grains and chloroplasts are still scattered throughout the pericarp. The outer walls of the cells of the epidermis of the ovary wall are slightly cutinized. In the mature caryopsis the pericarp and the integuments lie in close contact and can not be separated readily. The placenta, the nucellus just beneath the embryo, and the walls of the outer cells of the lemma and palea are lignified. The lemma and palea adhere very closely to the ripe caryopsis.

Up to the present time no differences have been found in the morphology or microchemistry of the membranes of the caryopses of these two species, which would account for the differences in the physiology of their germination.

TABLE 1.—*A summary of the microchemistry of the membranes of the caryopses of Poa pratensis and P. compressa*

Stage of development of the caryopsis	Where various substances were found in caryopsis at different stages of development			
	Pectic substances in cell walls of—	Cellulose in cell walls of—	Suberin and cutin	Lignin
1. Three large antipodals and a very small embryo.	Ovary wall, inner and outer integuments, and nucellus.	Inner cells of glumes.	Inner cell walls of inner and outer integuments slightly suberized.	
2. Caryopsis fully grown but green; aleurone cells formed but having thin walls.	Ovary wall and aleurone layer.	Ovary wall and inner cells of glumes.	-----do-----------------	
3. Caryopsis fully grown but green; aleurone cells having thick walls.	-----do-------------	Ovary walls, aleurone layer, and inner cells of glumes.	Inner cell walls of inner integument have formed a comparatively thin layer of suberin. Inner cell walls of outer integument heavily suberized. Outer cell walls of epidermal cells of ovary wall are cutinized.	Rachilla and cell walls of outer cells of lemma and palea.
4. Caryopsis mature; ovary wall compressed and shrunken.	Ovary wall, aleurone layer, and glumes.	Ovary wall, aleurone layer, and glumes.	Inner cell walls of inner integument have formed a comparatively thin layer of suberin. Inner cell walls of outer integument are heavily suberized. Outer cell walls of epidermal cells of ovary wall are cutinized.	Rachilla; cell walls of lemma and palea; nucellus just beneath embryo; placenta.

SUMMARY

In *Poa pratensis* and *P. compressa* the ovary wall is composed of four or five rows of parenchymatous cells the innermost row of which is the chlorophyll layer, which gives the characteristic green color to the ovary. The ovule is campylotropous. The inner and outer integuments are each composed of two layers of cells.

Four megaspores are formed. Usually the deepest but sometimes the outermost megaspore develops to form the embryo sac. Two megaspores often develop within the same nucellus, and each forms an embryo sac.

The usual eight-nucleate embryo sac is formed.

The antipodals are very large and persist until late in the endosperm formation when they become vacuolated and disappear. Usually three and not more than five antipodals have been observed in one embryo sac. If two embryo sacs are present within the same nucellus six or more large antipodals are present.

The endosperm nucleus divides and forms free nuclei in a peripheral layer of cytoplasm in the nucellar cavity. A parietal layer of cells is formed. The cells later divide and fill in the central cavity. The single layer of aleurone cells is differentiated when endosperm formation is practically complete.

The evidence presented indicates that polyembryony in *Poa pratensis* and *P. compressa* originates from two or more embryo sacs within the same nucellus. In some instances both embryos are

completely developed at maturity; in other instances one is fully developed and the other only partially developed. When two embryo sacs are within one nucellus, a peripheral layer of endosperm is associated with each embryo. Usually only one endosperm surrounded by the aleurone layer has been observed at maturity.

In the development of the embryo, growing points are found in succession. These are the primordia of a vegetative cone, coleoptile, and epiblast.

The coleoptile or plumule sheath is formed by the meeting of two outgrowths about the vegetative cone.

The primary radicle is not differentiated until the vegetative cone and coleoptile are well formed.

The mature embryo has a shieldlike scutellum with a poorly developed epithelial layer. The vegetative cone with its first and second leaves is surrounded by the coleoptile except for a small opening. The primary radicle terminates with a root cap; both the primary radicle and the root cap are surrounded by the coleorhiza. A procambium strand leads from the tissue between the plumule and the radicle into the upper part of the scutellum. An epiblast is present.

In the young ovaries of *Poa compressa* and *P. pratensis*, the cells of the ovary wall contain much starch. Their cell walls give a pectic reaction but later also contain cellulose. The outer cell walls of the epidermal cells become cutinized as the seed matures.

The cells of the inner and outer integument contain much starch. Soon after fertilization a layer of suberin is found in the cell walls of the inner integument adjacent to the nucellus, and in the cell walls of the outer integument adjacent to the inner integument. Usually the inner suberized layer develops first. With further development of the caryopsis these suberized layers increase in thickness. The outer one becoming thicker than the inner. The protoplasm and unsuberized cell walls of the outer integument disappear and are presumably absorbed. The cell walls of the inner integument begin to collapse by the time the caryopsis has reached its full size.

The cell walls of the young glumes contain cellulose. As the seed reaches maturity the cell walls also contain lignin and pectic substances.

The mature caryopsis consists of a fully developed embryo and endosperm surrounded by a single layer of aleurone cells which have thick walls, one or two rows of collapsed nucellar cells, modified integuments, and pericarp. The inner layer of the inner integument consists of a comparatively thin layer of suberin. Its remaining cell walls and their contents are compressed. The inner layer of the outer integument adjacent to the inner integument is composed of a thick layer of suberin. The remaining cells and their contents are presumably dissolved. The ovary wall cells are compressed into a thin layer. The outer cell walls of the epidermis are cutinized. The lemma and palea which adhere closely to the ovary contain lignin and pectic substances.

No differences have been found in the development of the embryo or of the membranes of the caryopses of *Poa compressa* and *P. pratensis*, which would account for the differences in the physiology of their germination.

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LIFE HISTORIES AND HETEROTHALLISM OF THE RED BREAD-MOLD FUNGI OF THE MONILIA SITOPHILA GROUP[1]

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INTRODUCTION

The red bread mold has long been known as a bakery pest and has caused much loss to bakers as well as to housewives. Its occurrence is somewhat erratic, but when a bakery becomes infested there is more or less continual trouble and loss until the fungus has been thoroughly eradicated.

Related species develop extensively on sugar-cane bagasse and this becomes a serious problem where this bagasse is used commercially as a basis for insulating board. The fungi of this group have also been found on silage and sometimes in connection with field, transportation, and storage rot of fruits, especially strawberries, raspberries, and apples, but appear to be of little economic importance in such cases. They are also well known to laboratory workers in mycology and pathology as a contamination in cultures of other organisms. The conidia are so light and powdery that they are easily and widely disseminated by the faintest breeze or movement of the air to which they are exposed.

HISTORICAL REVIEW

The common red mold appears to have been first reported in scientific literature by Léveillé (*37, p. 5, 6, 8*)[2] in France in 1843 under the name *Oïdium aurantiacum*. The same year it was also described by Montagne (*35, p. 377–378*) as *Penicillium sitophilum*. It was finally transferred to the genus Monilia by Saccardo. The fungus was called to the attention of Léveillé and Montagne by a commission (*37*) which was appointed by the French Minister of War in 1842 to investigate the cause of an infestation of the army bakeries which destroyed large quantities of bread. Another outbreak of the mold in bakeries in France was reported in Paris in 1871 by Decaisne (*9, 10*). De Claubry (*18*) also investigated this case and says that he had seen the same thing in bread in France in 1831. Decaisne states that sickness in one family whose members ate the moldy bread was attributed to the mold, but no evidence was found to support this. Outbreaks of the same fungus in bakeries have been reported by Murtfeldt (*36*) in Germany in 1917, by Mattirolo (*32*) in army bakeries in Italy in 1918, and by Herter (*20*) in Berlin in 1919. The same trouble is frequently reported from bakeries in this country, and only a few weeks ago cultures of the fungus were received from two different bakeries in New York.

In connection with the investigations of this fungus made in France, L. R. and C. Tulasne (*49*, *p. 90*) give an interesting account of its temperature relations, which being translated reads as follows:

Mauduyt of Poitiers, an expert in all branches of the natural sciences, in whose company we received our first knowledge of them, once thought that the dust [spores] of *Oidium aurantiacum* Lév. (*Penicillium sitophilum* Mont. in Ann. Sci. Nat. ser. II, t. xx, 1843, p. 377, pl. 16, f. 4, and Syllog. Pl. Crypt. 1856, p. 301), when mixed with ground meal and then baked in a hot oven, was damaged, so that if the bread afterwards went bad he thought the reason was to be found not at all in the included dust but in spores fallen from the air. He also tested some bread of the previous day which was everywhere much defiled by the *Oidium*, and when he had boiled it in water for half an hour it was never again spoilt by the contained mucedinous fungus or covered afresh by the red powder, but a piece of the same bread, when purified by similar boiling, could be readily infected by the seeds of *Oidium* falling from the air or purposely scattered over it, so that within two days it became completely red. (*Cf. Epist. ad Pictonum ediles* missam, Oct. 30, 1847.) The recent experiments of the famous L. Pasteur on the Mucedinei agree with the statements of the Poitevin naturalist. For they prove that the same spores of Mucedines, which could withstand without harm a heat of 120° or 125° for one hour or more in dry air or in a tube exhausted of air, were killed at once in boiling water; and the same thing happened with the dusty particles floating in the air, to which, falling perpetually, that author thinks all kinds of fermentation are due. (*Cf. Comptes Rendus*, t. LII., p. 16–19, Jan. 7, 1861.) All this agrees with what Turpin says about *Torula Cerevisiae* Turp. (in Walter Claubey's *Repert. Chim.* t. for 1838, p. 272, 279), for that fungus, as shown by Colin and Thénard, when placed for a little while in boiling water, lost at least for a time thereby any power of exciting the fermentation of a sugary liquid. Therefore the mode of action of the Gascons is seen to be contrary to the opinions of the learned, and we believe the only experiments on which reliance can be placed are those by which Payen asserted he had proved that the seeds of *Oidium aurantiacum* Lév., even when moistened retained their power of germinating uninjured, after being subjected to a temperature of 105° and 120°, so that the experimenter need not be surprised if bread made of contaminated wheat soon goes red on account of the Mucedine which it contains, since the inside of the loaf is not thought to be exposed in the oven to a heat of 100°. (*Cf. Comptes Rendus*, t. xxvii, p. 4–5, July 3, 1848.)

The mold in loaves of bread develops from spores contaminating either the flour after it has been milled or the wheat before it is ground. After the mold has been introduced into a bakery the dough from which the bread is made may also be contaminated by spores floating in the air of the bakery. According to recent experiments made by Tokugawa and Emoto (*48*), conidia of *Monilia sitophila* are killed in saturated steam at 100° C., although in a moist condition they are able to survive a temperature between 70° and 80° for five minutes, and in a dry atmosphere they are able to endure a temperature as high as 130°. There would be little probability, according to the statements of the authors just cited, of the conidia being destroyed in the loaf in baking, since records of baking temperatures made by the Bureau of Home Economics of this department show that when bread is baked in an oven at a temperature of 200° C. the temperature of the interior of the loaf does not exceed 95° to 98°. According to the temperature tests of the writers, conidia immersed in water and heated to 72° for four minutes are killed, and most of them are killed when subjected to a temperature of 65° for the same period. Moisture conditions, period of heating, and other factors enter so largely into the determination of the death point that it is difficult to tell whether or not the spores would be killed in ordinary baking.

Various physiological experiments with this mold have shown that it will cause fermentation and produce alcohol in the absence of

oxygen. It has also been demonstrated by Went (*51, 52*) that it produces 10 different enzymes as follows: Maltoglucase, trehalase, raffinase, invertase, cytase, diastase, lipase, tyrosinase, labenzym, and trypsin. Kunkel (*27, 28*) made a study of the toxicity of various inorganic salts upon the growth of this fungus. He found that of various nitrates tested potassium nitrate is least toxic and zinc most toxic. He also found that toxicity measurements made without regard to the inorganic substances in the culture medium are of little value.

In none of the literature relating to this fungus that the writers have been able to find is there mention of the finding of perithecia of *Monilia sitophila* either in nature or in artificial cultures. Went (*51*) in his physiological studies describes and illustrates the beginning of sclerotoid bodies resembling young perithecia. His only reference to them, however, is in the explanation of the plate in which he calls them "Hyphenknauel." They may have been the fundaments of sterile perithecia.

In 1901 Alfred Möller (*34, p. 294, pl. 2, fig. 34*), in his studies of tropical fungi, described a red mold found on corn bread and carbonized tree trunks, which he named *Melanospora erythraea*. In growing this fungus in culture he obtained an orange-colored mold which he did not attempt to name, but which according to his description and illustrations taken in connection with the writers' recent studies of the life history *of Monilia sitophila* was undoubtedly very closely related to this species, since the perithecial as well as the conidial forms are very similar. No one seems to have recognized heretofore the close relationship of Möller's conidial fungus to the common *M. sitophila*.

In 1924 Kitasima (*24, 25*), of Japan, published a report of observations which he had made upon an orange-colored mold found growing abundantly on trunks of trees which were burned during the great fire in Tokyo. He referred the fungus to *Monilia aurea* Gmel. Growing this in culture he obtained perithecia and ascospores of a fungus which he referred to the genus Anthostomella. An examination of Kitasima's fungus, which he sent the writers, shows that his conidial stage can not at present be distinguished from the common *M. sitophila*, and that the perithecial form, which he called Anthostomella, is really more nearly related to Melanospora as generally interpreted, and is evidently the same as that which the writers have obtained from *M. sitophila*.

LIFE HISTORY AND TAXONOMIC STUDIES

A few years ago Charles Thom, of the Bureau of Chemistry, in studying the organisms found on sugar-cane bagasse obtained cultures of a Monilia which produced both conidia and perithecia. Thom and Lathrop (*47*) in referring to this fungus say: "On the surface of the bales the first organism to develop was *Monilia sitophila*, which spread over the piles with great rapidity and draped them with festoons of orange-fruiting masses. Even after three to four weeks, handfuls of mycelium and spores could be gathered in many places." Cultures of this fungus furnished by Thom and Miss V. K. Charles, of the Bureau of Plant Industry, were studied and single ascospore cultures obtained which produced conidia very similar to typical *M. sitophila*.

A little later several cultures labeled *Monilia sitophila* were obtained from Westerdijk's culture collection at Baarn, Holland. One of these had been supplied by Stahel, of Dutch Guiana, another came from Herter, of Berlin, and another was originally grown by Bainier, of France. Much to the surprise of the writers, the culture from Dutch Guiana developed many perithecia, which were very similar in character to those in the cultures from Japan and Louisiana. Careful comparison, however, of perithecia, ascospores, and conidia shows that there are specific differences between them. These differences are shown by the descriptions and illustrations which follow.

A part of Möller's type of *Melanospora erythraea*, sent by H. W. Wollenweber, of Berlin, shows that it is different from the Surinam plant which the writers had at first thought might belong to the same species.

The association in cultures of conidia similar to those of *Monilia sitophila* and perithecia, which were referred by Möller (*34*) to Melanospora and by Kitasima to Anthostomella, naturally suggested a genetic relation between the two spore forms. In fact Kitasima (*25, p. 17*) says: "I have succeeded to get a pure culture derived from a single ascospore, produced the conidia which are of Monilia type in coloration, size, and shape." He does not state that perithecia appeared in this culture. The writers do not find, however, that anyone has reported the production of good perithecia in monosporous cultures of either conidia or ascospores and their early attempts were equally unsuccessful. This led naturally to the suggestion that the writers were dealing with a group of heterothallic species.

First efforts to grow single ascospore cultures were largely unsuccessful, owing to failure of the ascospores to germinate under ordinary conditions. It was soon found, however, that heating the spores caused them to germinate more readily. By pairing a large number of different ascospore or conidial cultures from different sources, it was demonstrated that certain paired cultures produced perithecia in abundance while others produced none. It was thus demonstrated that of the three species with which the writers have worked two are heterothallic, i. e., consist of two sexually different haplonts, and one species is homothallic. A full account of this phase of the work will be given in connection with the discussion of culture studies.

The discovery of another spore form of this mold may have important bearings upon the means of dissemination and the chances of reproduction of the fungus, and hence of its prevention and control. It is also important to know that there are several species which may infest bread, fruit, and other plant products and that they are heterothallic. If only one sex is present, the fungus should be easier to eradicate when it is found in a bakery or any other place, as no ascospores would be produced.

In attempting to determine the identity and systematic relationships of these species it was necessary to compare them carefully with the genera having similar perithecial forms as well as conidial stages. In the present state of our knowledge of the Pyrenomycetes it is difficult to define accurately and to limit the various genera that have been described. As at present treated, many genera include a heterogeneous group of species. It is therefore desirable to select some particular species of a genus as a basis for a concept of the group.

As already mentioned, two of the species with which the writers are concerned have been referred to different genera. Möller (*34*) referred his species to Melanospora, and Kitasima (*24*) referred his to Anthostomella, the genus to which *Melanospora destruens* (Shear) comb. nov. was first referred.

Melanospora was described by Corda (*7, p. 24*) in 1837. He included in it three species in the following order: *Melanospora zamiae, M. chionea*, and *M. leucotricha*, all of which he illustrated. The first species, *M. zamiae*, has not since been reported, as far as known, except for a specimen found on rice in India and so named by E. J. Butler. The other two species are very similar and apparently congeneric. The life history of neither of these is satisfactorily known. Zukal (*53, p. 538*) grew *M. leucotricha* but obtained no conidia or pycnidia. The ascospores germinated from one end only. In accordance with present usage and in the interest of stability and uniformity in nomenclature it seems best to accept *M. chionea* (Fr.) Cda. (pl. 1, A), the commonest and best known species of Corda, as the nomenclatorial type of Melanospora, *M. chionea* has membranous, gray perithecia, with a long slender beak which is fimbriate at the apex. No conidia are known. The perithecial form of *Monilia sitophila* differs from this in the color and character of the perithecia, in the absence of a distinct elongate fimbriate beak, and in its persistent asci.

Anthostomella, to which Kitasima (*25*) referred the perithecial form of his Monilia, is really less nearly related to this fungus than Melanospora and several other closely related genera. Anthostomella was described by Saccardo (*40, p. 84*). *Anthostomella limitata* Sacc., the first of the three species originally placed in the genus by the author, is apparently a good representative of the group as generally accepted. The perithecia in this species are submembranous, gregarious, entirely embedded in the tissue of the host, and covered with a more or less blackened clypeus or epistroma. Good paraphyses are also present and no conidial form is known.

Ceratostoma of Fries (*15, p. 337*) is another related genus described in 1818 with 35 species. *Ceratostoma (Melanospora) chionea* was included as No. 16 of the list. In 1849 Fries (*16, p. 396*) revised his idea of the genus and included but six species, specifying particularly *C. chionea* as the type of the genus. In a footnote he makes the following statement (translated):

> When I collected *S. chionea* I immediately considered it to be the type of a new genus = Ceratostoma Obs. Myc. II; then I saw a very close relationship to various beaked Sphaerias which could be in no way distinguished. For this reason I reduced them to Sphaeria in the S. M., and moreover the limits are changing, for Sphaerias No. 110–112 should be placed here with equal justice. Meanwhile following the suggestion of recent authors, I separated this and the following subgenera, at the same time predicting a close connection with Sphaeronaema; for species of this genus having a globose perithecium sharply distinct from a beak can not be distinguished from Ceratostoma. The name Melanospora Cord. is suitable for only the first species, therefore, we have fixed upon the older name to be retained.

It is clear from this that he regarded his genus as a synonym of Melanospora of Corda. The usage of later authors, however, as Fuckel (*17, p. 127*), Schroeter (*42, p. 312*), Saccardo (*41, p. 215*), and Lindau (*29, p. 406*) has changed the application of the name by retaining Melanospora for *M. chionea* and similar species, and

applying Ceratostoma to a group of species of which *C. notarisii* Sacc. may be designated as the nomenclatorial type. In adopting this interpretation Lindau (*29, p. 406*) says in a footnote (translated):

As Schröter has shown, Fries's concept of the genus does not correspond with the present application of the name. The species he recorded as belonging to it must be referred to Melanospora. If one follows priority *Ceratostoma* must replace *Melanospora*, and a new name be given the present group of species. This would only make confusion. I therefore accept the present usage.

According to this interpretation of Ceratostoma the writers' species differ from it in having neither a carbonous, long rostrate perithecium nor a true persistent paraphyses and in having a Monilia conidial stage.

Included in Sordaria and Podospora are certain species which show many points of relationship with the fungi treated here. Some of these species are known to have conidia of the Spicaria type, e. g., *Sordaria montanensis* Griffiths (*19, p. 49, pl. 19, fig. 13*) and *Podospora brassicae* Brefeld (*5, p. 196–198, pl. 6, figs. 1–2*). The same thickened ring is also found in the apex of the ascus in some species of these genera. *Ascotricha pusilla* (E. and E.) Chivers (*6, p. 220, pl. 17*) and some species of Chaetomium also show close relationship.

Certain species referred to Rosellinia, Sphaeroderma, Erythrocarpa and Sphaerodermatella are also nearly related and are in need of further study and comparison before the natural boundaries and relationships of these genera can be determined.

As far as known, the nearest relative of the new genus Neurospora, to which the red bread-mold fungus is being referred, was described by Vincens (*50*) as *Melanospora mangini* (pl. 2, F). This has very similar perithecia and the ascospores have anastomosing ridges. It also has a conidial stage which the author refers to Spicaria (pl. 2, F, *d*). Several other species referred to Melanospora have known conidial forms which are referred to Spicaria. These are evidently more closely related to the new genus Neurospora than to true Melanospora. Whether they should be transferred to this new genus or not must depend upon further knowledge of the systematic value of the ascospore characters and the conidial stage. The conidial forms of other species of Melanospora have been referred to Scopulariopsis and Acmosporium. Another group of species which has been referred to Melanospora may be represented by *M. destruens* (Shear) (*43; 44, p. 43*) (pl. 1, B; pl. 3, D). This group differs from the typical Melanospora as reeprsented by *M. chionea* in having thicker subcoriaceous perithecia and short, nonfimbriate ostioles.

Small globose bodies resembling sclerotia and known as bulbils, which have been found associated with cultures of species referred to Melanospora and are placed in the form genus Papulospora as treated by Hotson (*22*), usually develop in monosporous cultures of the heterothallic species of Neurospora, and sometimes occur in the homothallic species and in the cultures from the paired haplonts. Berkhout (*2, p. 39*) also reports such bodies in her cultures of *Monilia sitophila*. Whether these are the fundaments of sterile or aborted perithecia or a peculiar form of asexual body can not be stated at present.

The genus Monilia, to which the conidial stages of Neurospora are here referred, was first described by Hill (*21, p. 69*) in 1751. In this
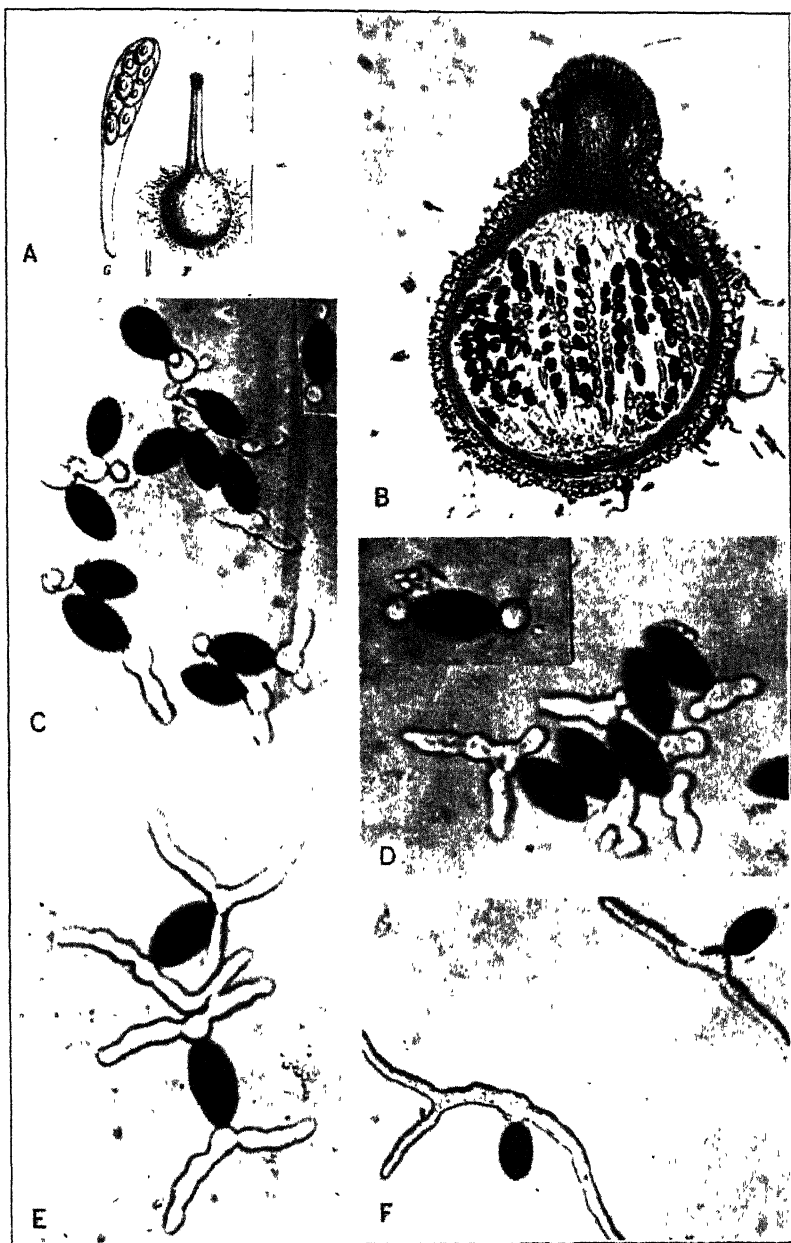
Photomicrographs of Melanospora and Neurospora: A, Perithecium and ascus of *Melanospora chionea* (after Winter); B, section of perithecium of *M. destruens;* C-F, germinating ascospores; C. *Neurospora sitophila;* D, *N. crassa;* E, *N. tetrasperma;* F, *Melanospora destruens.* B× 148, C-F × 368
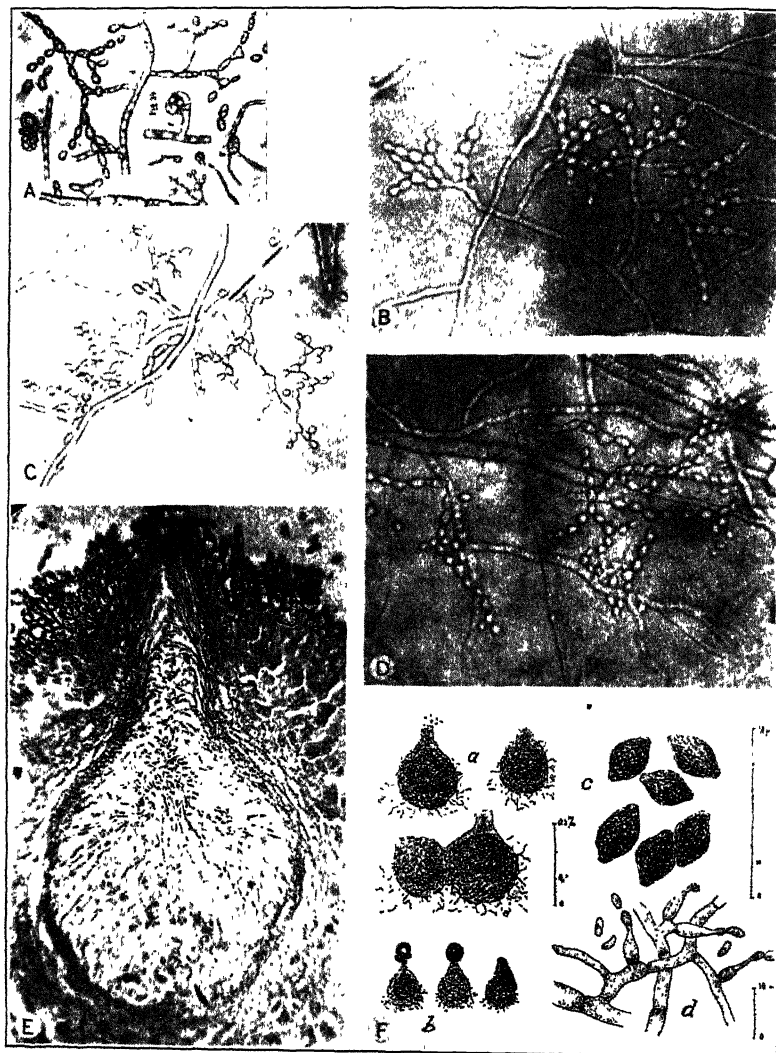
Conidia and perithecia of Neurospora and Melanospora: A, Conidia of *N. erythraea* (after Moller); B, *N. sitophila;* C. *N. crassa;* D, *N. tetrasperma;* E, section of young perithecium of *N. crassa* showing cavity filled with degenerating hyphae resembling paraphyses; F, perithecia, *a, b*; ascospores, *c*, and conidia, *d*, of *Melanospora mangini* (after Vincens). A–D × 185. E × 216

connection it is interesting to note that he makes a statement which it has taken nearly 200 years to verify.   His statement is as follows:

> The Monilia all produce distinct male and female flowers.   The male flowers are antherae, placed on a short stamina, on the summit of the pedicle, or near the summit, surrounding it in form of a fine powder.   The female flowers we are able to distinguish nothing of, except the seeds, which are arranged together in series by means of a glutinous matter, and stand sometimes close to one another, sometimes more distinct.

This was written during the period when the question of sex in flowering plants was being much discussed by botanists.   Hill was one of those who having accepted sex in plants thought it should be true of fungi as well as flowers; hence his rather naïve attempt to distinguish male and female flowers in Monilia.

Hill's conception of the genus was based upon Micheli's (*33, p. 212, tab. 91*) descriptions and figures of Botrytis and Aspergillus. The present so-called form genus Monilia is not usually credited to Hill because he was not a binomialist, and because of his broad and too indefinite application of the name.

The name is usually attributed to Persoon (*38, p. 691*), who first used it in 1801 to include 12 species.   These were as heterogenous as those of Hill.   The original species of Persoon have gradually been separated and most of them placed in other genera.   According to present usage, *Monilia fructigena* Pers., the conidial stage of *Sclerotinia fructigena*, might well be taken as the nomenclatorial type of Monilia.   If the names of conidial stages were to be restricted to those having the same generic perfect stage it would be necessary to refer *M. sitophila* to another form genus.   It does not seem desirable, however, to follow such a course, as the inconvenience and change would be much greater than any benefit which might be derived.   Until all the conidial forms have been connected with their perfect stages it will be necessary to retain these form genera to include conidia and pycnidia which have great morphological similarity but different life histories or perfect stages.

The results of the investigations of *M. sitophila* reported here show that the conidial forms of several species of Ascomycetes have heretofore been included under this name.   As will be seen from an examination of the descriptions which follow, the differences in the conidia of the different species are comparatively slight, and can be determined only by careful cultural studies made in connection with the perithecial stages.   According to our present knowledge the synonomy of *M. sitophila* (Mont.) Sacc. is as follows: *Penicillium sitophilum* Mont.; *Oidium aurantiacum* Lév.; *Monilia martini* Ell. and Sacc.; *Oospora aurantiaca* (Lév.) Herter; *Monilia aurantiaca* (Lév.) Herter not Peck and Saccardo.; *Oidium lupuli* Matth. and Lott. (*31, p. 86–87*); *Oospora lupuli* (Matth. and Lott.) Lindau (*30*); *Monilia aurea* Kitasima not Gmelin; (?) *Monilia carbonaria* Cke.

As the result of the life history and morphological studies of the red bread-mold fungus and its near relatives a new genus, Neurospora, and three new species are described.

Neurospora gen. nov.

Perithecia gregarious or scattered, smooth or with loose hairs, subcoriaceous to subcarbonous; ostiole papillate or short rostrate, perithecial cavity lysigenic, filled at first with parallel, septate hyphae (paraphyses?) which begin to collapse and disappear as soon as the young asci start and are entirely gone when the asci

are full grown (pl. 2, E); spores hyaline to yellowish-brown at first, becoming black or greenish black when mature, continuous, longitudinally ribbed (pl. 3, A). Conidial stage of the *Monilia sitophila* type (pl. 2, B).

The perithecia are either superficial or embedded when grown on agar media; when superficial they usually show a loose growth of weak hyphae on the surface. The crowded septate hyphae which fill the perithecial cavity at the time the asci start are doubtfully described as paraphyses. They disappear so soon that they would not be observed except in very young perithecia.

Neurospora sitophila sp. nov.

Conidial stage (*Monilia sitophila* Mont.): On corn-meal agar tufted or irregularly pulvinate at first, soon becoming effuse and fluffy; conidial masses varying from pale salmon to orange color; mycelium hyaline, creeping, septate; sporogenous hyphae irregularly ascending, septate, dichotomously branched; conidia catenulate, connected by a narrow isthmus when mature, globose to subglobose, smooth, pale salmon to orange in mass, 10 to 12μ diameter, mostly 10 to 11μ (pl. 2, B).

Perithecial stage: Perithecia gregarious or scattered, superficial or immersed on agar media, smooth or loosely soft, hairy, brown to black subcoriaceous, 200 to 300μ diameter; ostiole papillate; asci cylindrical, short stipitate, apex with a thickened gelatinous ring, 8-spored, $140-160 \times 12-14\mu$ paraphyses (?) crowded, multiseptate, filling the perithecium at first, collapsing and disappearing as the asci develop; ascospores overlapping uniseriate, elliptical, with 16 to 17 sometimes branched longitudinal ribs, olivacious becoming dark greenish black, $20-26 \times 10-15\mu$, mostly $23-25 \times 13-15\mu$ (pl. 2, A; pl. 3, A).

Type: Selected, culture No. 5574, slide No. 5575, from which Plate 4, A, was taken and in mycological collections, Bureau of Plant Industry. Original from Herter, Berlin.

The description is based on cultures on corn-meal agar in test tubes. Whether this species is identical with Montagne's fungus it is impossible to determine at present, as the ascospore stage of his plant was not found. This is the common red bread mold of Europe and America and presumably Montagne's (*35*) species; for practical purposes, therefore, this name may be adopted.

Neurospora crassa sp. nov.

Conidial stage (*Monilia crassa* nom. nov.): On corn-meal agar tufted or irregularly pulvinate at first, soon becoming effuse and forming fluffy aerial masses at the sides of the tube; pale salmon to light orange. Mycelium scanty, creeping, septate; sporogenous hyphae, irregular, ascending, septate, dichtomously branched; conidia catenulate, connected by a narrow isthmus, globose to subglobose when mature, smooth, yellow to pale orange in mass, 6 to 8μ diameter, mostly 6 to 7μ (pl. 2, C).

Perithecial stage: Perithecia gregarious or scattered, subglobose, superficial or immersed when grown on agar, smooth or loosely soft hairy when superficial, yellowish brown at first, becoming blackish brown, thick walled, subcoriaceous, 400 to 600μ diameter; ostiole obtuse, conical, black; asci cylindrical, short stipitate, with a thick gelatinous ring at the apex, 4-spored, $150-175 \times 18-20\mu$; paraphyses (?) crowded, multiseptate, filling the perithecium at the time the asci appear, collapsing and disappearing as the asci develop, entirely lacking in the mature perithecium; ascospores overlapping-uniseriate, elliptical, with about 20 longitudinal, sometimes branched ridges, olivaceous at first, becoming dark brown to black, $23-32 \times 11-16\mu$, mostly $27-30 \times 14-15\mu$ (pl. 4, B; pl. 3, B). This species is heterothallic.

Habitat: On bagasse, Marrero, La. From Charles Thom, of the Bureau of Chemistry. Known only from this material.

Type: Culture No. 5576, slide No. 5577, from which Plate 4, B, was taken. In mycological collections, Bureau of Plant Industry.

On corn-meal agar in Petri dishes the conidial masses form about the sides of the dish and are about the same color as in *Monilia sitophila*, but smaller in quantity than in that species when grown on the same medium and under the same conditions. The same difference occurs in the cultures but is less striking. On bread in flasks the conidial growths are very similar.
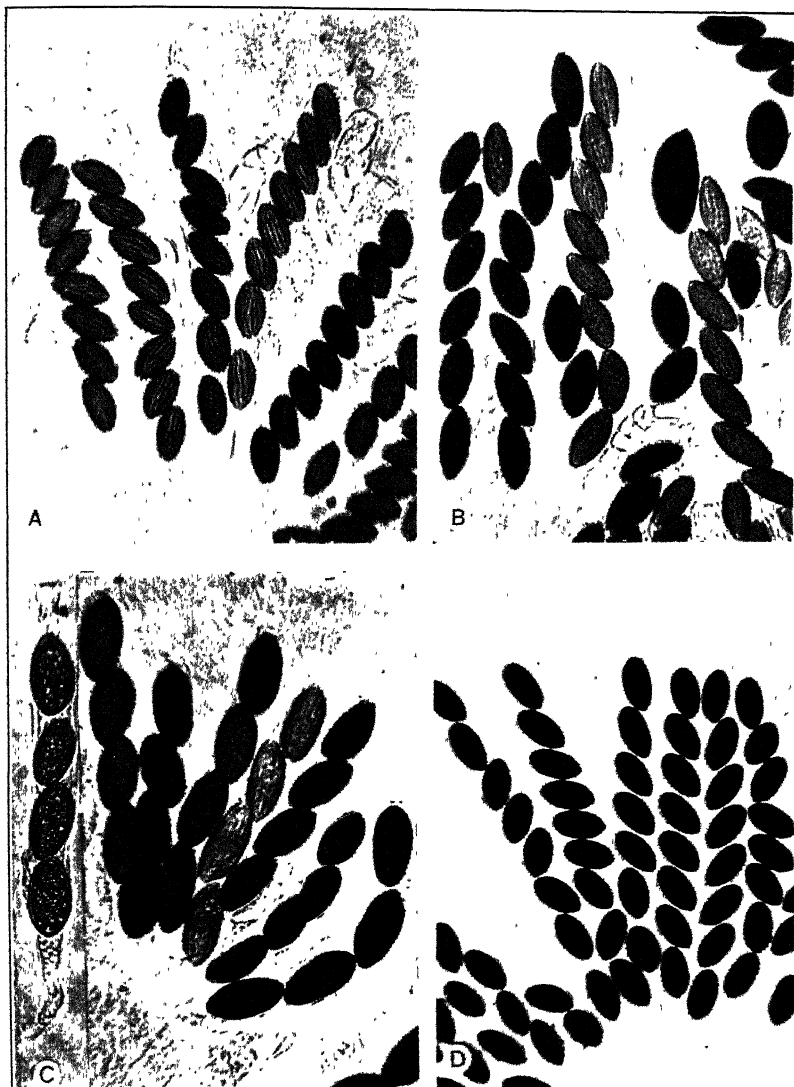
Photomicrograph of asci and ascospores of Neurospora and Melanospora: A. *N. sitophila;* B, *N. crassa;* C, *N. tetrasperma;* D, *Melanospora destruens* × 332
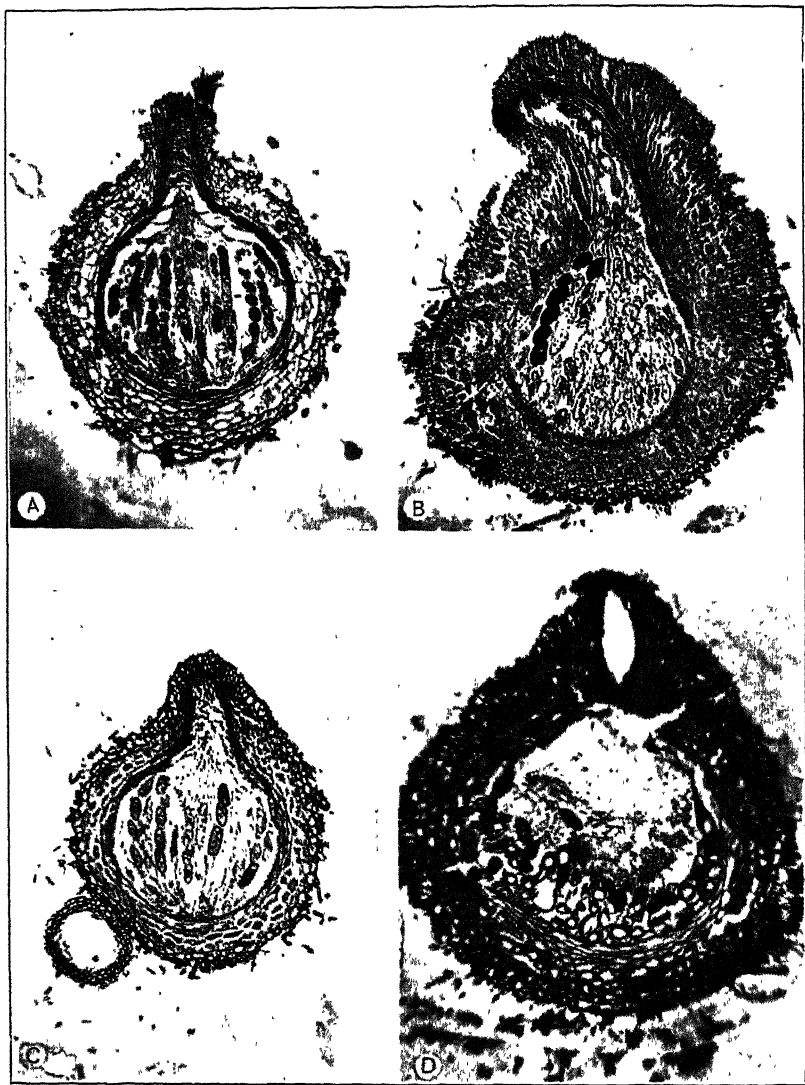
Photomicrographs of sections of perithecia of four species of Neurospora: A, *N. sitophila;* B., *N. crassa;* C, *N. tetrasperma;* D, *N. erythraea.* ×126

Neurospora tetrasperma sp. nov.

Conidial stage (*Monilia tetrasperma* nom. nov.): On corn-meal agar in tubes very scanty whitish growth of mycelia about the upper edge of the agar slant; conidial tufts usually small and scanty, pale salmon; sporogenous hyphae irregularly ascending, septate, dichotomously branched; conidia catenulate, connected by a narrow isthmus when mature, globose to subglobose, smooth, nearly hyaline or pale salmon in mass, 8–11μ in diameter, mostly 8–9μ (pl. 2, D).

Perithecial stage: Perithecia gregarious or scattered, superficial or immersed when grown on agar media, smooth or loosely and sparsely soft hairy when superficial, yellowish when young, becoming dark brown to black; 250–300μ in diameter; wall medium thick, subcoriacious; ostiole obtuse, papillate; paraphyses (?) crowded, septate, collapsing,and disappearing as soon as the asci appear; ascospores uniseriate or somewhat overlapping, oblong-elliptical with about 20 longitudinal, sometimes branched ridges, olivaceous at first, becoming dark brown to black, 29–35×14–16μ, mostly 30–31×15μ (pl. 4, C; pl. 3, C). This species is homothallic.

Habitat: Substratum unknown, Surinam, Dutch Guiana, Stahel.

Type: Culture No. 5578, slide No. 5579 from which plate 4, C, was taken. In mycological collections, Bureau of Plant Industry.

On bread the conidia are formed in abundance and have the same general character and appearance as *M. sitophila*.

The cultures of this came originally from Gerald Stahel, Government mycologist, Parimaribo, Dutch Guiana, and were sent the writers from the Central bureau voor Schimmelcultures at Baarn, Holland. These cultures when received contained perithecia.

Neurospora erythraea (Möll.) comb. nov.

Conidial stage (*Monilia erythraea* comb. nov.): Tufts abundant bright orange color in mass; fertile hyphae dichotomously branched, conidia catenulate globose to subglobose, smooth, 7 to 11μ diameter, mostly 8 to 9μ (pl. 2, A).

Perithecial stage: Perithecia gregarious, superficial subglobose loosely and sparsely soft hairy, yellow at first becoming reddish and finally dark brown, thick walled, subcoriacious, 400 to 600μ diameter; ostiole obtuse, short conical; asci cylindrical, stipitate, gelatinous ring at apex, 4-spored, about 250μ long; paraphyses lacking in mature perithecia; ascospores overlapping uniseriate, elliptical with 20 to 22μ longitudinal somewhat branched ridges, yellowish at first, becoming greenish black to black, 28–38×13–18μ, mostly 34–36×15–16μ. This species is apparently homothallic (pl. 4, D).

Habitat: On corn bread and burned stumps of "Rocas" Blumenau, Brazil, A. Möller (*34, p. 294*).

Type: No. 190 (5580). Slide No. 5581. From Möller's collection in alcohol in Botanical Museum, Berlin. In mycological collections, Bureau of Plant Industry.

The above description and Plate 2, D, were made from a part of Möller's original culture sent by H. W. Wollenweber of Berlin, which shows only the perithecia with 4-spored asci. Möller also mentioned finding perithecia with 8-spored asci associated with similar conidia. The 8-spored form was found on a burned stump of orange and was thought by him to be merely a form of *Neurospora erythraea*. It is more probable, however, that this was a distinct.species.

Having no living material of Möller's plant, the writers can not prove that it was homothallic. The fact that he obtained perithecia in cultures made by the transfer of a bit of hyphae and that the writers' 4-spored species is homothallic would indicate the probability that such is the case in *Neurospora erythraea*. This species is distinguished from *N. tetrasperma* by its much larger perithecia and ascospores.

HETEROTHALLISM IN THE ASCOMYCETES

The discovery of heterothallism in the Mucorineae by Blakeslee (*4*) and the work on heterothallism in the higher basidiomycetes inaugurated by Mlle. Bensaude (*1*) and Kniep (*26*) need not be reviewed

here. Proof that species of ascomycetes are also heterothallic has been produced in but few cases. The work of Edgerton (*14*) has been frequently cited as showing that *Glomerella cingulata* is heterothallic. Edgerton makes no such claim in his paper. He distinctly states that both the strains produced perithecia when grown alone. However, when the same two strains were grown together on opposite sides of a Petri dish culture more perithecia were produced along the line where the mycelia met than when the strains were grown separately. This is a well known phenomenon. Many instances have been cited in the literature showing that bacteria or other fungous contaminations as well as different colonies of the same fungus often have a beneficial effect in changing the constitution of the medium or in otherwise stimulating the production of ascocarps. The erroneous idea seems to prevail that when fruiting bodies are produced along a line where two mycelia meet this is an indication of heterothallism.

Ascobolus magnificus was the first ascomycete to be definitely proved by the combination of monosporous cultures to be heterothallic (*13*). Only certain pairs of such cultures gave rise to ascocarps. Monosporous mycelia always remained sterile. This species produces functional ascogonia and antheridia which appear to rise from different hyphae. No sex organs are produced in monosporous cultures. In all probability the gametophytes come to maturity and produce their reproductive structures only when grown together in the same medium, the mycelium of each sex so changing the nature of the medium as to stimulate the development of reproductive structures on the mycelium of the opposite sex.

Kirby (*23*) has reported that *Ophiobolus graminis* is heterothallic, ascocarps being produced only when cultures are grown together in certain combinations, monosporous cultures remaining sterile. Kirby believes that the fertile strains are sexually differentiated. Davis (*8*), working with monosporous cultures of the same fungus originally isolated by Kirby, finds that ascocarps are developed in abundance from monosporous cultures. He has repeatedly isolated other single spore cultures of the same species which likewise developed ascocarps. Davis finds that strains from Arkansas and Kansas could not be induced to sporulate. Kirby in recent correspondence with the writers as a result of further work has modified his published statements regarding the heterothallism of this fungus. In view of the fact that Davis states that this Ophiobolus is homothallic and not heterothallic as claimed by Kirby, it is clear that further study on this species is necessary before the question can be considered settled.

Derx (*11*) working with species of Penicillium has proved conclusively that *Penicillium luteum* is heterothallic. Several single ascospore cultures were isolated and grown in all possible combinations. He found that about half the ascospores were of one sex and half the other.

Betts (*3*) has proved beyond a doubt that *Ascobolus carbonarius* is also heterothallic. He grew cultures from seven spores from a single ascus and found that three of them belonged to one sex and four to the other.

CULTURE STUDIES

The material used for cultures has been obtained from different countries, as noted in part in connection with the discussion of the three species of Neurospora described above. This material turned out to be of two different kinds. In all cases each culture had developed the Monilia stage more or less luxuriantly at the time it was received. A few of the cultures had developed ascocarps in transit; one culture produced perithecia later when transferred to a more suitable medium. Each collection was at first designated either by a name, number, or source without particular regard to its specific identity, which was in most cases not determined until later, as will be explained in connection with Table 6. It may be said here that with the exception of Nos. 1, 2, and 15, all of these cultures were later referred to *Neurospora sitophila*. A list of the cultures used, together with their history as far as known to the writers at the time this work was done, follows:

1. *Neurospora crassa.* The material for the work with this form was obtained from Charles Thom. It has been made the type of *N. crassa.* It was originally obtained in 1924 from sugar-cane bagasse near New Orleans.

2. S 227. A culture sent in by J. E. Flynn, Cornell University, who obtained it from H. H. Whetzel.

3. Herter. This material was obtained from the Centraalbureau voor Schimmelcultures at Baarn, Holland. It came originally from Herter and is presumed to be the same material studied by him. It has been made the type of *Neurospora sitophila.* The culture developed mature ascocarps and an abundance of conidia.

4. Japanese. The culture containing ascocarps and conidia received from Doctor Kitasima, Tokyo, Japan. The culture was labeled "single ascospore."

5. Arlington. The culture of the fungus which appeared as a contamination in Petri dish cultures in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. After transfers to corn-meal agar in test tubes this material developed ascocarps as well as conidia.

6. B. P. I. A culture obtained from a contamination in a Florence flask containing fruit juice in a laboratory at the Department of Agriculture, Washington, D. C.; appeared about the same time as No. 5, the Arlington culture, and may have been originally from the same source. Ascocarps and conidia developed on the fruit juice in the flask.

7. Scales. A culture originally sent by F. M. Scales, of Mount Vernon, N. Y., obtained by him from a bakery in New York City. The culture as originally received produced a great abundance of conidia. A large number of subcultures obtained by transfer from the original material to corn-meal agar developed ascocarps.

8. Bainier. A culture obtained from Thom, as representing the Bainier material kept in culture in l'Ecole de Pharmacie, at Paris. This culture developed conidia and numbers of small sclerotiumlike bodies, but no ascocarps.

9. B 704. Culture obtained from the laboratory of H. H. Whetzel, who had originally received it from Wooster, Ohio, produced only conidia.

10. Rose. A culture which developed only conidia. Contributed by D. H. Rose, who had obtained the fungus from rotting strawberries at Chadbourn, N. C.

11. Columbia University. A culture from Columbia University. According to the history of the culture it was originally obtained from a bakery in St. Louis, Mo., by Kunkel (*28*).

12. Berkhout. A culture which developed conidia and numbers of very small bulbils or sclerotia was a part of the material reported on by Christine Berkhout (*2*).

13. Kauffman. Material contributed by C. H. Kauffman, Ann Arbor, Mich., produced only conidia.

14. Cooley. A culture obtained by J. S. Cooley from an apple-receiving room in a cold-storage plant at Charlottesville, Va. The same fungus had been obtained repeatedly from the air in apple-packing sheds.

15. *Neurospora tetrasperma.* A culture obtained from the Centralbureau voor Schimmelcultures at Baarn, Holland. The culture was originally contributed

by G. Stahel from Surinam. The culture had developed large numbers of ascocarps when received and only a very few conidia. Like *N. erythraea*, the species described by Möller (*34, p. 294*), each ascus develops only four spores.

SPORE GERMINATION

The conidia of the Monilia stage germinate readily under all ordinary cultural conditions. According to the statements of Tokugawa and Emoto (*48*) and others, the conidia when in a fairly dry condition resist for 15 or 20 minutes temperatures considerably above the boiling point of water.

The writers have found that in case of the *Monilia sitophila* there are a number of factors that must be considered in determining the effect of heat on germination. When the conidia are thoroughly wet most of them are killed when heated for five minutes at 65° C. and all are killed when kept at 72° C. for the same time. Should a little air be caught in a tangle of conidia and fragments of hyphae, some of the conidia will not be wet even though immersed, and these will survive when heated at much higher temperatures. It has also been found that, as reported by Thom and Ayers (*46*), heating of the conidia delays their germination sometimes for several days, so that the observations should be made for a period of at least 10 days after the experiment has been made.

In this connection Thom and Ayers in studying the effect of Pasteurization on mold spores found that some of the conidia of *Aspergillus fumigatus*, for example, germinated after having been floated on the surface of milk in a test tube and heated for 30 minutes at 65.6°C. These authors found that nearly all of the conidia of the different molds tested were killed when immersed in milk at 68.3° for 30 seconds.

The question as to whether or not conidia in flour survive the baking process would depend on whether they are thoroughly wet in mixing the dough, how porous the dough is after rising, and whether they start to germinate before being heated. The size of the loaf, the temperature of the oven, the length of time the loaf is baked, and the rapidity with which the loaf is cooled are some of the factors that must be taken into account.

Another important factor not heretofore considered is the presence of ascospores of the fungus. As will be pointed out later, the ascospores are much more resistant to heating under moist conditions than are the conidia. Experiments are being carried out in cooperation with L. H. Bailey of the Bureau of Chemistry to determine more definitely the effect of various temperatures and conditions of baking upon the viability of spores contained in the dough.

For the study of heterothallism the conidia alone could not serve as the primary source of pedigreed cultures. The ascospores rarely germinate under ordinary culture conditions. Möller (*34, p. 294*) was unable to obtain germination of ascospores of *Neurospora erythraea*. Kitasima (*25*) evidently had great difficulty in securing cultures from ascospores. Any attempt to obtain pedigreed cultures from spores which so rarely germinate under ordinary conditions would be a very tedious process and would often result in contaminations by conidia of the species. In order to secure numerous ascospore cultures it was necessary to find some method of insuring germination of the ascospores.

The method of germinating ascospores of certain species of Ascobolus described by Dodge (*12*) and further tested out by Ramlow (*39*) and Betts (*3*) was tried out and found to be highly satisfactory. The ascospores are sown on the surface of an agar medium in a Petri dish and spread out so that the spores may be picked out separately later. The Petri dish is then set in any ordinary drying oven where the temperature can be raised gradually from room temperature to 90° C. The thermostat may be so regulated as to require 20 or 30 minutes to do this. The exact temperature to which the spores are raised is not of vital importance. It will be found that a large percentage of ascospores so treated will begin to germinate within four or five hours. Transfers of single germinating spores must be made within a few hours, since the germ tubes grow rapidly and tend to become interlaced with those from other spores. A spore usually germinates from a pore in each end. A rather large globule is first extruded and from this vesicle usually two germ tubes develop and grow out in opposite directions (pl. 3, A, B, C).

When quantities of conidia are carried over with the ascospores in making the sowings, it is advisable to allow the cultures to stand a few hours so that the conidia may begin to germinate. The heating necessary to stimulate ascospore germination will kill the germinating conidia and facilitate the isolation of single ascospores. A better way is to use only material in which the perithecia are beginning to discharge the spores, which are shot some distance. The bottom of a Petri dish containing agar may be inverted over the culture bearing the perithecia so as to catch the ascospores as they are shot from the fruit bodies. In this way very few conidia will be carried up to the agar and the ascospores will be scattered far enough apart on the surface of the agar to be removed separately with little difficulty after germination. The percentage of germination is ordinarily high when the spores have been properly heated. The higher the temperature within the limits given above the more satisfactory will be the results of attempts to obtain cultures free from conidial contamination.

It has been found that corn-meal agar is an excellent medium for this culture work. Conidia are not produced in such abundance on this medium as on some others. While the three species studied produce perithecia under proper conditions when grown on corn-meal agar in Petri dishes, it is much better to use tube cultures. It goes without saying that in working with a fungus which produces conidia in such masses certain precautions are necessary to avoid contaminations, especially when material from many different sources is to be handled.

NEUROSPORA CRASSA

Following the culture methods described above, over 100 single ascospore cultures have been obtained. These were supplemented by several single conidium cultures, and mixed cultures derived from several ascospores were also made for comparison.

Svedelius (*45*) and others are now using the term "haplont" to distinguish a particular plant of the gametophytic phase of an alga or fungus. In the case of such a fungus as Neurospora, the gametophytic growth originating from a single ascospore is capable of

reproducing itself indefinitely by fragments of the hyphae or by the formation of conidia. The mycelium or haplont derived in this way would be analogous merely, but not homologous, to a clon in the higher plants. While no one is now misled by such statements as growing, pairing, or mating cultures or strains in different combinations, it is simpler in this connection to refer to monosporous cultures as haplonts.

The monosporous mycelia (haplonts) at first produced only conidia. After about 10 days some of them began to form structures which in size and color look like young perithecia. Such sclerotium-like bodies resembling the bulbils of certain species of the form genus Papulospora have been noted by several persons who have worked with *Monilia sitophila*, as already mentioned. These bodies were observed for several months but none developed ascospores. Several hundred subcultures were made by transferring the tips of hyphae or by transferring conidia; no perithecia were produced in these cultures. The mixed cultures, however, which were derived from three or four ascospores, usually developed perithecia within two weeks.

The cultures originally derived from single ascospores have been grown in different combinations. The results obtained by pairing 15 different haplonts in all possible ways, and in duplicate, are tabulated so that the cultures fall into two groups. (See Table 1.) The marginal and top numbers are used to designate particular cultures. Failure to produce perithecia is indicated by the minus sign; a positive sexual reaction and the formation of normal perithecia is indicated by the plus sign. The table shows that any mycelium in one group when grown with any other mycelium of the same group produces only conidia; when a mycelium from one group is grown with any mycelium from the other group perfect perithecia as well as conidia are matured. The results of this work prove conclusively that *Neurospora crassa* is heterothallic.

TABLE 1.—*Monosporous mycelia or haplonts, from random selection of ascospores of Neurospora crassa; haplonts paired in all possible ways and in duplicate* [a]

	1	4	5	8	16	19	2	3	6	7	9	10	11	12	20
1	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
4	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
5	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
8	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
16	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
19	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
3	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
9	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
11	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
12	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
20	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

Each ascus of this species contains eight spores which are shot violently from the ascus at maturity. Occasionally the eight spores so discharged fall in a separate group. They can then be removed separately and germinated. Five sets of such haplonts, each set

consisting of eight monosporous mycelia derived from spores from the same ascus, were made. The haplonts from each set were then grown in all possible combinations. The results obtained with each of the five sets agree. Table 2 shows the results of mating the mycelia in one set. The marginal and top numbers represent the haplonts from single spores picked up at random after they had been discharged from a single ascus. The results show clearly that four spores from an ascus belong to one sex and four to the other.

TABLE 2.—*Eight monosporous mycelia or haplonts of Neurospora crassa. Spores from the same ascus, picked up at random after their discharge. All combinations* [a]

	1	2	6	7	3	4	5	8
1	−	−	−	−	+	+	+	+
2	−	−	−	−	+	+	+	+
6	−	−	−	−	+	+	+	+
7	−	−	−	−	+	+	+	+
3	+	+	+	+	−	−	−	−
4	+	+	+	+	−	−	−	−
5	+	+	+	+	−	−	−	−
8	+	+	+	+	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by —. A positive sexual reaction and the formation of normal perithecia is indicated by +.

NEUROSPORA SITOPHILA

Ascocarpic material of *Neurospora sitophila*, as typified by the Herter material referred to above, has been received from five different sources. The first and most extensive culture work with this species was done with material collected at Arlington, Va. Ninety-six cultures were obtained by germinating single ascospores. These mycelia were grown in several thousand cultures on different media, singly and in different combinations. Monosporous mycelia produce only conidia and in most cases numerous sclerotiumlike bodies. The 96 mycelia were not grown in all possible combinations, but it was proved conclusively that the Arlington material is heterothallic. The results obtained by later cultures of the Herter and Japanese material, which are the only results presented here in tabulated form, substantiate those previously obtained.

A number of haplonts derived from single ascospores of the Herter material have been grown for several months, both alone and in all possible combinations. The results obtained by growing in all possible combinations eight of such mycelia chosen at random are shown in Table 3. The cultures used may be arranged in two groups. Perithecia are produced only when a mycelium from one of the groups is grown together with a mycelium from the other group. For example, mycelium 4 develops only conidia when grown alone or in combination with either mycelium 2 or 6. When, however, haplont 4 is grown with either mycelium 1, 3, 5, or 8, both conidia and perithecia are developed. The Herter material of *Neurospora sitophila* is also heterothallic.

The culture of the Japanese material of *Neurospora sitophila* was maturing perithecia when received. Twenty haplonts or monosporous cultures from ascospores were obtained and subcultures from these

have been grown for nearly a year. In no case has such a culture produced perithecia. Eleven of these haplonts were grown in combination, as shown in Table 3. This shows that the Japanese material of *N. sitophila* is likewise heterothallic. The haplonts fall into two groups. Any mycelium from one group will produce perithecia when grown with a mycelium from the other group. Even though all the cultures of one group be grown together no perithecia are formed.

TABLE 3.—*Monosporous mycelia (haplonts) obtained by random selection of ascospores of Neurospora sitophila (from Herter). All combinations* [a]

	2	4	6	1	3	5	7	8
2	−	−	−	+	+	+	+	+
4	−	−	−	+	+	+	+	+
6	−	−	−	+	+	+	−	+
1	+	+	+	−	−	−	−	−
3	+	+	+	−	−	−	−	−
5	+	+	+	−	−	−	−	−
7	+	+	+	−	−	−	−	−
8	+	+	+	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

The original culture from Doctor Kitasima was according to the label, obtained from a single ascospore. Without denying that a culture from a single ascospore of this material might for some very definite reason develop ascocarps, the writers merely wish to state here that all of their single-spore cultures from this material produce only conidia and sclerotiumlike bodies when grown alone. Table 4 shows the results of mating haplonts from these cultures.

It is unnecessary to describe in detail the results obtained by growing large numbers of monosporous cultures of this species from ascosporic material received from the other three sources mentioned. The results prove beyond question that the species *Neurospora sitophila* is heterothallic and that single-spore cultures so far obtained have not produced perithecia.

As stated above, the original material from each of the five sources contained both of the elements necessary for sexual reproduction of the species. Frequently, however, mycologists find that cultures of *Monilia sitophila* obtained from rotting fruit, from bakeries, or from burned trees produce only conidia, regardless of what culture medium is employed. A number of such cultures of the Monilia stage have been received within the present year. In some cases hundreds of subcultures were grown in attempts to obtain ascocarps, but without success. It became a matter of much interest to determine whether perithecia could be obtained by mating two or more of these forms together in a culture. The results of this work, discussed further in connection with Table 6, show that cultures may be classed in two groups, based on their sexual reactions. All but one of the conidial cultures received may be referred to the species *Neurospora sitophila* or a species so similar to it morphologically as to be indistinguishable from it.

TABLE 4.—*Monosporous cultures, obtained by random selection of ascospores of Neurospora sitophila (Japanese). All combinations* [a]

	1	3	5	8	9	12	2	4	6	10	11
1	−	−	−	−	−	−	+	+	+	+	+
3	−	−	−	−	−	−	+	+	+	+	+
5	−	−	−	−	−	−	+	+	+	+	+
8	−	−	−	−	−	−	+	+	+	+	+
9	−	−	−	−	−	−	+	+	+	+	+
12	−	−	−	−	−	−	+	+	+	+	+
2	+	+	+	+	+	+	−	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−	−
6	+	+	+	+	+	+	−	−	−	−	−
10	+	+	+	+	+	+	−	−	−	−	−
11	+	+	+	+	+	+	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

It may be of interest to know, for example, that *Monilia sitophila* obtained from rotting strawberries at Chadbourn, N. C., forms perithecia when grown together with the *M. sitophila* with which Berkhout (*2*) worked in Holland. Both collections produce only conidia when grown separately. The culture from a St. Louis bakery (*28*) and the one from the Bainier collection do not develop perithecia when grown separately or together, but each develops perithecia when paired either with the *M. sitophila* received from a laboratory at Ann Harbor, Mich., or with a culture sent in by Bunzel from a bakery in New York City.

<div align="center">NEUROSPORA TETRASPERMA</div>

As already stated, the culture originally obtained from Doctor Stahel from Surinam had matured large numbers of perithecia when received. The species is characterized especially by the fact that the asci, with few exceptions, contain only four spores. The species produces very few conidia in culture on corn-meal agar, but when grown on bread or such a medium as potato-dextrose agar great quantitites of conidia are formed, so that in the conidial stage it would not be readily distinguished from the red-mold stage of *Neurospora sitophila*. Growing the two species side by side on corn-meal agar, however, would enable one to distinguish readily the Surinam species, which has been described above under the name *N. tetrasperma*.

A number of monosporous cultures were obtained by isolating single ascospores from the heavy spore print formed on the tube of the original culture. Within six or seven days each of these cultures showed mature ascocarps which were discharging spores. This proved conculsively that *Neurospora tetrasperma* is commonly homothallic.

Over 200 additional monosporous mycelia obtained from other ascosporic subcultures of this species have developed perithecia on corn-meal agar.

Möller (*34, p. 294*) noted that in *Neurospora erythraea* the ascospores varied considerably in size. The average size, he stated, was about 36μ long. As observed previously under the discussion of this species, it has been found that the ascospores of the Surinam material, *N. tetrasperma*, average about 30 to 32μ long. These measurements apply to spores from an ascus bearing 4 spores. Careful examinations

of crushed preparations show that occasionally an ascus develops 5 or 6 spores, but more rarely 3, 2, or only 1 spore. In case an ascus develops 5 spores 2 of them will be very small, while the other 3 will be of normal size. An ascus with three spores ordinarily will have 1 spore which is about 40 to 45μ long. One-spored asci are very rare. In the 2 or 3 that have been found the spores were from 60 to 65μ long. The smallest spores are about 20 to 25μ long. Over 50 monosporous cultures of the abnormally large spores have been made. One has no difficulty in distinguishing these large spores from the common type. With the exception of two somewhat doubtful cases which need further study, the results show that each of the unusually large spores possesses all the elements necesary for sexual reproduction and produces good perithecia.

In the case of the very small spores it has been found that the size varies considerably so that it is not always possible to distinguish these small spores definitely from the others. Forty-four monosporous cultures from spores which were judged to be under size were isolated. Only five of these cultures developed perithecia. The others developed large numbers of very small sclerotiumlike bodies and a few conidia. The cultures that remained sterile were then grown in all possible combinations. The results of growing 21 of these haplonts together is shown in Table 5. It will be seen that the 21 cultures fall into two groups. There are 9 cultures in one group and 12 in the other. Mycelium from one group produces perithecia only when grown in combination with mycelium from the second group. In other words, this species which is ordinarily homothallic occasionally develops spores which are not totipotent. Each spore contains only one of the sex elements. By selecting at random the small spores, haplonts may be obtained which when grown in combination develop normal perithecia with asci which produce predominantly only four spores. The results of all the culture work with this species show that regardless of whether perithecia are obtained by growing what may be called the normal spores, or by growing those that are especially large or very small, the resulting perithecia and ascospores are of the same type.

TABLE 5.—*Small spore selections of Neurospora tetrasperma (commonly homothallic) which are heterothallic* [a]

	1	3	D	8	30	32	E	H	J	2	4	6	7	10	28	29	31	33	34	G	5
1	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
3	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
D	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
8	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
30	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
32	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
E	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
H	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
J	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
28	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
29	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
31	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
33	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
34	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
G	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−

[a] Marginal and top numbers refer to particular cultures. Failure to produce perithecia indicated by −. A positive sexual reaction and the formation of normal perithecia is indicated by +.

IDENTIFICATION OF CULTURES BEARING ONLY CONIDIA

Certain cultures which developed only conidia of the Monilia stage are listed on page 1029. In their conidial stage the two heterothallic species are not readily distinguished with certainty. The most practical way by which the species affiliations of the sterile cultures could be determined was to grow them in combination with mono-sporous cultures of known origin. If, for example, a culture of unknown identity be mated with each of two reciprocal haplonts of *Neurospora crassa*, and if in one case perithecia are produced similar to those normally developed by *N. crassa*, such a result would indicate that the unknown culture probably belongs to that species. In Table 6 the reciprocal haplonts of a species or those which when paired will produce perithecia are designated for convenience as A and B without regard to their sex, which is unknown at present. The unknown strains were grown in combination with several different pairs of reciprocal haplonts of each of the two species *N. crassa* and *N. sitophila*. The results shown in Table 6, as previously stated, prove that with one exception each unknown was one or the other sex of *N. sitophila*.

PERITHECIA RESULTING FROM MATING HAPLONTS OF DIFFERENT SPECIES

For purposes of experiments on crossing or hybridization one may consider as heterothallic the small spore selections of the homothallic species, *Neurospora tetrasperma*, discussed in connection with Table 5. Pairs of reciprocal haplonts of each of the three species and all of the sterile cultures of unknown identity noted above were grown in all possible combinations. These results are also included in Table 6. In case no perithecia were produced the result is indicated as before with the minus sign. When the haplonts mate and perithecia result from the combination this is indicated by the plus sign, and the strains crossed are assumed to belong to the species having a perithecium and spores of the same kind. The results of crossing reciprocal strains or haplonts of one species with reciprocal strains of another species are indicated by the minus sign when no sexual reaction occurred, and by the letter X when the reaction results in the formation of ascocarps with mature spores. In these cases the course of development of the perithecia and ascospores differs from that of either species. There are various differences in the form and size of the spores, and the lack of uniformity in reaching maturity is such as would be expected in hybrids.

TABLE 6.—*Haplonts of unknown identity and those known to belong to Neurospora sitophila (from various sources), N. crassa (Thom coll.), and N. tetrasperma (Stahel coll.), paired in all possible combinations* [a]

	Herter A	Japanese A	Arlington A	B.P.I.A	Scales A	Bainier	B 704	Rose	Columbia University	N. crassa A	N. tetrasperma from—tetrasperma A	Herter B	Japanese B	Arlington B	B.P.I.B	Scales B	Berkhout	Kauffman	Cooley	S 227	N. crassa B	N. tetrasperma from—tetrasperma B
N. sitophila:																						
Herter A	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
Japanese A	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
Arlington A	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
B. P. I A	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
Scales A	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
Unknowns:																						
Bainier	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
B 704	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
Rose	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
Columbia University	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	X	X	X
N. crassa A	−	−	−	−	−	−	−	−	−	−	−	X	X	X	X	X	X	X	X	+	+	X
N. tetrasperma A	−	−	−	−	−	−	−	−	−	−	−	X	X	X	X	X	X	X	X	X	X	+
N. sitophila:																						
Herter B	+	+	+	+	+	+	+	+	+	X	X	−	−	−	−	−	−	−	−	−	−	−
Japanese B	+	+	+	+	+	+	+	+	+	X	X	−	−	−	−	−	−	−	−	−	−	−
Arlington B	+	+	+	+	+	+	+	±	+	X	X	−	−	−	−	−	−	−	−	−	−	−
B. P. I. B	+	+	+	+	+	+	+	+	+	X	X	−	−	−	−	−	−	−	−	−	−	−
Scales B	+	+	+	+	+	+	+	+	+	X	X	−	−	−	−	−	−	−	−	−	−	−
Unknowns:																						
Berkhout	+	+	+	+	+	+	+	+	+	X	X	−	−	−	−	−	−	−	−	−	−	−
Kauffman	+	+	+	+	+	+	+	+	+	X	X	−	−	−	−	−	−	−	−	−	−	−
Cooley	+	+	+	+	+	+	+	+	+	X	X	−	−	−	−	−	−	−	−	−	−	−
S 227	X	X	X	X	X	X	X	X	X	+	X	−	−	−	−	−	−	−	−	−	−	−
N. crassa B	X	X	X	X	X	X	X	X	X	+	X	−	−	−	−	−	−	−	−	−	−	−
N. tetrasperma B	X	X	X	X	X	X	X	X	X	X	+	−	−	−	−	−	−	−	−	−	−	−

[a] In this table A and B are reciprocal as to sex reaction. The minus sign indicates that no perithecia were produced; the plus sign indicates that normal perithecia developed; the letter X indicates that hybrid perithecia with mature asci were formed.

As noted above the table shows that with the exception of S 227 all of the cultures received which were sterile with regard to asco-carps when grown alone, belong to the species *Neurospora sitophila.* The culture S 227 formed perfect perithecia when grown with haplont A of *N. crassa,* so that it is known to belong to the species *N. crassa,* whose perithecium has the same characters. After these results were obtained and the history of the culture further determined it was found that Whetzel originally received the culture from Thom, who had also contributed the material for the culture work conducted by the writers on *N. crassa.* In making the transfers from Thom's cultures which were producing perithecia, only the B strain was sent to Whetzel. At least the culture S 227 with which the writers did their work remained sterile as long as it was cultured alone.

Any haplont A of the species *Neurospora crassa* and a haplont B of *N. sitophila* when mated develop a few large perithecia which

mature a relatively small number of ascospores. A positive reaction occurs when the heterothallic haplonts A of *N. tetrasperma* are mated with haplonts B of either *N. crassa* or *N. sitophila*, resulting in the development of perithecia with ascopores.

At this writing no culture work has been done with spores from perithecia which may be true sexual hybrids between the species; that is, the ascocarps which are developed when strains of different species are crossed may readily be produced as the result of unions between reciprocal haplonts. Further work will be necessary before any conclusion can be reached concerning the nature of these structures. The results of cytological work on Neurospora will be published in another connection.

SUMMARY

The red bread-mold fungus, *Monilia sitophila* Mont., was first described in 1843. It has frequently been found seriously infesting bakeries in Europe and America. It has also been reported on various fruits and tree trunks and stumps recently killed by burning, and on sugar-cane bagasse.

This study shows that instead of one species there are at least four which have been passing under this name. The life histories of these four species have been determined.

Instead of belonging to Sclerotinia, as might be expected from the great resemblance of the conidia to those of *Monilia cinerea*, the ascospore stage is found to be a pyrenomycete which seems to belong to an undescribed genus, which is named Neurospora.

The conidia of three species grow readily at ordinary laboratory temperatures. When exposed to a temperature of 120° C. for 30 minutes dry conidia are killed; when wet they are killed when exposed to a temperature of 72° for 5 minutes. The ascospores are more resistant when dry.

The ascospores do not germinate under ordinary cultural conditions, but grow readily after heating in a moist condition at 65° to 70° C. for a few minutes.

Baking tests show that 1-pound loaves in which have been placed both conidia and ascospores and which have been baked in an oven for 35 minutes at 215° C. do not develop mold.

Bakeries contaminated with these fungi can be freed from them by thorough cleaning of walls, floors, and utensils with live steam, soap and water, formaldehyde, or any good antiseptic.

Two of the species, *Neurospora sitophila* and *N. crassa*, have been found to be heterothallic. *N. tetrasperma* is usually homothallic, but occasionally heterothallic haplonts have been obtained by the selection of abnormal spores. *N. erythraea* has not been studied in culture by the writers, but is probably homothallic. Cultures from single spores of the heterothallic species produce only conidia. Reciprocal haplonts when mated produce perithecia.

Crossing the different species by mating their haplonts results in the formation of perithecia with ascospores which seem to have hybrid characters.

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A STUDY OF THE TOXICITY OF ACID LEAD ARSENATE ON THE JAPANESE BEETLE (POPILLIA JAPONICA NEWM.) [1]

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INTRODUCTION

During the past few years lead arsenate has been recommended for the control of the Japanese beetle. The results of its use show that this arsenical has given very satisfactory protection to the foliage, which may be attributed to the repellent action of acid lead arsenate on the beetle. The habits of the beetles are such that no accurate figures have been obtained on the percentage killed. The results of a large number of experiments, involving different methods, indicate that the kill is approximately 20 per cent of the beetles occurring on the sprayed foliage. These results, however, give no indication of the quantity of arsenic required to kill the beetle, or the quantity of sprayed foliage which it would have to eat to cause death. As a step to the solution of these problems the experiments reported in this paper were undertaken.

EXPERIMENTAL PROCEDURE

For several reasons it has been difficult to determine the value of a spray in the field. Of these, the following are important: (1) Many beetles which consume a lethal dose of arsenic probably leave the sprayed foliage before dying; (2) many beetles are repelled from sprayed foliage before they have taken a toxic dose; and (3) the beetles are incessantly active. For these reasons it seemed necessary to carry on the experiments in cages, so that the beetles might be kept under observation.

PREPARATION AND APPLICATION OF SPRAY

Suspensions of lead arsenate in water were prepared from samples of dry commercial lead arsenate containing 32 per cent, by weight, of arsenic oxide, as determined by analysis. Six mixtures of lead arsenate were used, varying progressively from 1 to 6 pounds of it to 50 gallons of water. To the mixture was added a little lime and casein mixture, to facilitate the spreading of the lead arsenate evenly over the surface of the foliage. Care was taken to use the smallest proportion of the lime and casein mixture consistent with satisfactory results, which proved to be 3.9 grams to 3 gallons of the mixture. To determine the various quantities of arsenic oxide (As_2O_5) remaining

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 11
June 1, 1927
Key No. K-191

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on the foliage, the leaves of several smartweed plants were first measured with a planimeter, and the plants were then dipped into one of the mixtures of lead arsenate and lime and casein mixture. As soon as the leaves were dry they were clipped off the plant and allowed to fall into a wide-mouthed bottle, which was then securely corked. The organic matter of the leaves was subsequently oxidized in the collecting bottle with sulphuric and nitric acids, and the arsenic oxide contained determined by a standard procedure. This method was used for each of the six mixtures and gave for each the total quantity of arsenic on leaves of known area. Table 1 shows the quantity of arsenic oxide per square inch of leaf surface, for the six mixtures of lead arsenate and lime and casein mixture used in the tests, and its equivalent expressed in terms of lead arsenate. The square inches, to a convenient fraction of a unit, could be read, leaf by leaf, from the planimeter. Since arsenic oxide constitutes 32 per cent, by weight, of the commercial arsenate used, the quantity of such arsenate equivalent to each determination of the oxide was readily found by multiplying by 3.125.

TABLE 1.—*Arsenic oxide, and its equivalent in lead arsenate, per unit area of leaves sprayed with mixtures of lead arsenate, lime and casein mixture, and water*

Quantity of lead arsenate per 50 gallons of water	Number of leaves measured and dipped	Leaf surface measured (both sides)	Quantity of arsenic oxide (As_2O_5) found in sample	Quantity of lead arsenate ($PbHAsO_4$) found in sample [a]	Arsenic oxide (As_2O_5) per square inch of leaf surface	Lead arsenate ($PbHAsO_4$) per square inch of leaf surface
Pounds		*Sq. in.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	32	204.46	1.6461	5.1441	0.0081	0.0252
2	35	195.90	4.5317	14.1616	.0231	.0723
3	34	209.16	9.0725	28.3516	.0434	.1355
4	54	340.94	16.5238	51.6369	.0485	.1515
5	57	375.26	19.5211	61.0034	.0520	.1626
6	35	181.88	10.0939	31.5434	.0555	.1734

[a] The lead arsenate is the chemical equivalent (in terms of the commercial lead arsenate used) of the arsenic oxide (As_2O_5) determined by analysis.

A comparison of the quantities of arsenic per unit area adhering to the smartweed foliage dipped into the mixtures containing various proportions of lead arsenate shows that the increase in arsenic is rather uniform to and including the third mixture. The difference in quantity of arsenic oxide adhering to the foliage in the case of that mixture and of the next is very slight. This similarity of effect may account for the results of the control spraying in the field, practically as good protection being obtained when the lead arsenate was used at the rate of 3 pounds to 50 gallons of water as when it was used at the rate of 4 pounds to the same quantity of water.

MEASUREMENT OF QUANTITY OF FOLIAGE EATEN

Potted smartweed plants, averaging 12 inches in height and free from injury, were dipped into the various suspensions of lead arsenate and lime and casein mixture. Great care was taken that all parts of a plant should be thoroughly wetted with the mixture. A small cage, 16 inches high and 6 inches in diameter, constructed of galvanized-wire gauze having 16 meshes to the inch, was placed over

each plant and a beetle introduced. The wire cage was firmly pressed down into the soil about the base of the plant, to prevent the escape of the beetle. These cages were kept in an outdoor open-type insectary, and several tests were made with them, an equal number of cages being used to each mixture, and four check cages. Equal numbers of male and of female beetles were used. No starvation tests were made, experiments having shown that beetles starved for 48 hours or more will feed readily upon poisoned foliage.

Hourly observations of the foliage eaten, the time of feeding, and the mortality of the beetles, were made only between the hours of 8 a. m. and 5 p. m. The greater part of the feeding was done during the period of observation, but some of it was carried on outside of this period. The fact that a number of beetles died while not under observation made it necessary to give the time in the tables as approximate, instead of as absolute. If a beetle was feeding at the time the observation was made, it was allowed to continue its feeding and a record was made of the fact. In many cases the beetle was in the soil at the time of observation; when this occurred, the beetle was dug up in order to determine whether it was living or dead. Many of the beetles were found dead in the soil about the base of the plant. The cages containing the poisoned foliage were kept in use until the observations were terminated by the death of the beetles, whereas the cages containing unsprayed foliage were discontinued as the beetles in them died, or as soon as all the experiments on poisoned foliage were ended. When a portion of a leaf had been eaten the leaf was clipped from the plant and a photographic print of it was made immediately, showing the actual size of the leaf and the area eaten. The area eaten and the background of the print were shown in black, and the remaining, uneaten area appeared white. In many cases where the area eaten was too small to be measured with a polar planimeter, it was enlarged 100 times in area by the use of a projectoscope. This apparatus projected the print on a screen, over which was traced the enlarged area. These areas could then be measured accurately by the polar planimeter and reduced by mathematical calculation to the corrected measurements.

CRITERIA OF TOXICITY

In order to judge the value of the methods described and the results obtained by using them, the following factors, impossible to control, are enumerated: (1) The beetles were gathered in the field at random, and varied in size and in age; (2) individual beetles vary as to quantities of arsenic necessary to kill; (3) some beetles die immediately after eating a lethal quantity of arsenic, whereas others are sick several days before dying; (4) the weather and the degree of hunger influence the time of feeding and the quantity consumed; (5) the distribution of arsenic on the foliage is somewhat uneven; and (6) the growth of the leaf during the experiment produces areas unprotected by arsenic. On account of these uncontrollable factors the results are valuable only for a rough comparison. In computing the toxic dose required to kill it was assumed that in all cases the arsenic oxide was evenly distributed over the foliage.

Table 2, based on several tests in each series, is a condensed summary of averages of the calculated quantity of arsenic required to kill, of the time from the beginning of the experiment to the death of the beetle, of the amount of leaf area eaten, and other important data. The results tabulated indicate how effective arsenic oxide may be as a toxic agent in the field, if its repellent action to the beetle can be overcome. The areas eaten were so small that for purposes of comparison it seemed better to express them in square millimeters rather than in the original units of measurement with the planimeter. The conversions were made by multiplying the areas in square inches by 645.1606; and in the case of each of the six mixtures of lead arsenate and lime and casein mixture, the quantity of arsenic oxide and of lead arsenate per beetle was found by multiplying the quantity per square inch of leaf surface by the area in square millimeters of leaf surface eaten, and dividing the product by 645.1606.

TABLE 2.—*Comparison of data obtained from the feeding of individual Japanese beetles on unsprayed smartweed foliage, with data obtained for beetles feeding on smartweed foliage sprayed with mixtures containing various proportions of lead arsenate*

Quantity of acid lead arsenate to 50 gallons of water	Number of beetles observed	Approximate average time from beginning of experiment to first feeding	Approximate average time from first feeding to death	Approximate average time from beginning of experiment to death	Average area of leaf surface eaten per beetle [1]	Average quantity of arsenic oxide eaten per beetle	Average quantity of lead arsenate eaten per beetle
		Hours	*Hours*	*Hours*	*Sq. mm.*	*Mgm.*	*Mgm.*
1 pound_____	23	28	54	82	90.44	0.0011	0.0035
2 pounds_____	34	21	67	88	73.26	.0026	.0082
3 pounds_____	31	20	66	86	74.20	.0050	.0156
4 pounds_____	33	23	48	71	63.32	.0048	.0149
5 pounds_____	31	20	64	84	38.46	.0031	.0097
6 pounds_____	17	26	42	68	48.10	.0041	.0129
Control (unsprayed)	33	22	_____	_____	[2] 2,405.16	_____	_____

[1] The term "leaf surface" means the total surface of the upper and lower sides of the leaf. It does not mean the size of the hole made in the leaf.
[2] Not to be compared with sprayed foliage.

The data in Table 2 indicate that the area of leaf surface eaten per beetle tends to decrease as the arsenic content of the leaf surface increases. The average toxic dose, as shown by the table, varies from 0.0011 mgm. to 0.0050 mgm. of arsenic oxide (As_2O_5) and from 0.0035 mgm. to 0.0156 mgm. of lead arsenate ($PbHAsO_4$). The approximate average time from the beginning of the experiment to the first feeding varies from 20 hours to 28 hours. The approximate average time from the beginning of the experiment to the death of the beetle varies from 68 to 88 hours. The data indicate that a small percentage will die within 48 hours after the beginning of the experiment, and that only a small quantity of available arsenic will be needed to kill the beetle. Twenty-three beetles ate, before death occurred, an average of 90.44 sq. mm. of leaf area, sprayed with lead arsenate in the proportion of 1 pound to 50 gallons of water. Similarly, before dying, 34 beetles ate an average of 73.26 sq. mm. of leaf area, sprayed with lead arsenate at the rate of 2

pounds to 50 gallons of water; 31 ate an average of 74.20 sq. mm. of leaf area sprayed with 3 pounds to 50 gallons; 33 ate an average of 63.32 sq. mm. of leaf area sprayed with 4 pounds to 50 gallons; 31 ate an average of 38.46 sq. mm. of leaf area sprayed with 5 pounds to 50 gallons; and 17 ate an average of 48.10 sq. mm. of leaf area sprayed with 6 pounds of lead arsenate to 50 gallons of water. In the check cages, containing unsprayed foliage, 33 beetles ate an average of 2,405.16 sq. mm. of leaf area, only 3 beetles dying before the experiments were discontinued. However, the beetles in these cages fed for a longer time than did those in the cages containing the poisoned foliage.

SUMMARY

Various mixtures of lead arsenate were sprayed on smartweed and tested against 169 individual beetles. Observations were made as to the length of time of feeding, the area of foliage consumed, and the quantity of arsenic which caused death. The results indicate that small dosages of available arsenic are lethal, and that in case of ordinary infestations control in the field will be obtained without great injury by beetles to sprayed foliage. The protection afforded by a spray of lead arsenate, as shown by the small quantity of foliage eaten, is the outstanding fact established as a result of these experiments.

THE DEVELOPMENT OF THE PECAN NUT (HICORIA PECAN) FROM FLOWER TO MATURITY [1]

By J. G. WOODROOF, *Assistant in Horticulture*, and NAOMI CHAPMAN WOODROOF, *Assistant in Botany, Georgia Agricultural Experiment Station* [2]

INTRODUCTION

The increasing popular demand for information on the several regions or structures of the pecan nut (*Hicoria pecan* Brit.), their relative rate of growth, and the dates of the critical periods of their growth has indicated the need for a thorough study of the developing nut. The present paper is intended to supplement previous papers on morphological and physiological development of the buds and flowers of the pecan tree.

Stuckey (*5*)[3] studied 25 varieties of pecans during the flower stage and immediately following. His object was to study self-sterility in pecans and incidentally to note the morphological development.

The senior writer (*6*) studied the morphological development of the staminate flowers and briefly noted the development of the nut.

In a previously published article (*7*) the writers describe the time of fruit-bud differentiation and the subsequent development of the flowers. It was intended that such a study would lay an adequate foundation for a study of the nut itself.

Billings (*2*) studied fertilization in *Carya olivaeformis* Nutt. (*Hicoria pecan* Brit.). He found that the pollen tube passes down the axial tissue of the style to a point near the cavity of the ovary, where it turns and passes down the ovary wall close to the margin of the cavity. When a point is reached a little below the funiculus, the pollen tube curves toward the center, and when under the ovule turns upward to the embryo sac.

Nawaschin (*3*) described the method of fertilization in *Juglans regia*. He concluded that the pollen tube did not enter the micropyle but passed down the ovary wall and entered the ovule through the chalaza.

METHODS

Field notes and free-hand sections have been made annually for four years, and material has been collected for embedding in paraffin and sectioning with a microtome for two years. The first year embedding material was collected at 10-day intervals from the Alley and Frotscher varieties; the second year embedding material was collected every five days from the Alley and Nelson varieties. Material from the Stuart, Jerome, Alley, and Nelson varieties was taken twice each day for post-pollination studies. In the early stages the entire nut was embedded. As it grew larger only sections

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 11
June 1, 1927
Key No. Ga-5

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through the nut were used, and when the nut was almost grown only certain regions were embedded.

The same 25 varieties used by Stuckey and Woodroof (*5, 6*) were used for field notes and observation in this work.

Picric acid killing and fixing agent was found most satisfactory. Juel's fixative was satisfactory during the early stages but failed to fix the material well in later stages of growth. Carnoy's fixative caused shrinkage of the tissue and rendered it brittle. Flemming's weak fixative rendered the material too brittle to section well. Haidenhain's iron-alum haematoxylin was used for staining.

EXPERIMENTAL DATA

After pollination the surface of the stigmas become darkened and shrivel immediately. The entire stigmatic tissue, which extends to the region of
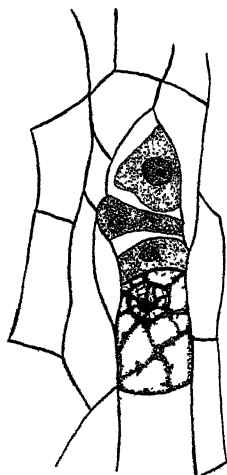
FIG. 1.—Four-megaspore stage, Stuart variety pecan, 18 hours after pollination. The three upper megaspores show indications of disintegration; the lower megaspore will develop into an embryo sac. × 830
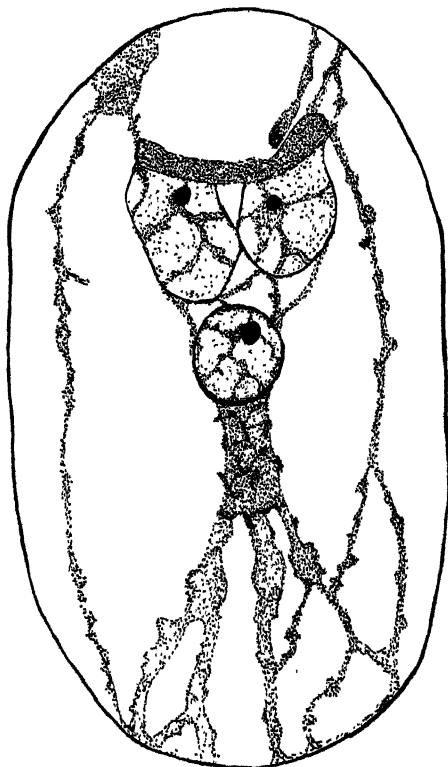
FIG. 2.—Mature egg apparatus, Alley variety pecan, June 7. × 830

the hull and shell, dies and becomes hard within two weeks after pollination. It is soon detached from the hull and shell regions and may drop off, or it may be held in place indefinitely by the vestiges of the bracts.

To determine the course of the pollen tube has been found to be difficult. The largest number of germinating pollen grains appear on the outermost surfaces of the stigmas, and the early course of the pollen tube is inward toward the vascular bundles of the stigma. The pollen tube apparently follows this series of vascular bundles until it reaches the chalaza. The writers have not followed the course of the pollen tube in detail, but indications are that it reaches the embryo sac through the chalaza.

Beginning at pollination time, about April 26 to 31, the condition of the embryo sac, embryo, and pollen tube at subsequent dates are summarized in Table 1:

TABLE 1.—*Condition of embryo sac, embryo, and pollen tube at different periods, beginning at time of pollination*

Time	Condition of embryo sac and embryo	Condition of pollen and pollen tube
At pollination	4 megaspore stage	Pollen shed.
12 hours after pollination	3 megaspores disintegrating; lower megaspore developing (fig. 1).	Pollen germinating.
24 to 40 hours after pollination	3 megaspores disintegrating. Lower megaspore undergoes nuclear division.	Pollen tube growing.
2 to 5 weeks after pollination	Nuclear division in embryo sac; 8 nuclei formed. Antipodals disintegrate.	Do.
5 to 7 weeks after pollination	Mature egg apparatus. Probable fertilization with formation of embryo (fig. 2).	
9 weeks after pollination	Well-defined endosperm; 1 cell embryo, attached at top of cavity of embryo sac (fig 3).	
10 weeks after pollination	2 to 4 cell embryo	
11 weeks after pollination	Embryo 8 to 16 cells	
12 weeks after pollination	Embryo 32 to 64 cells	
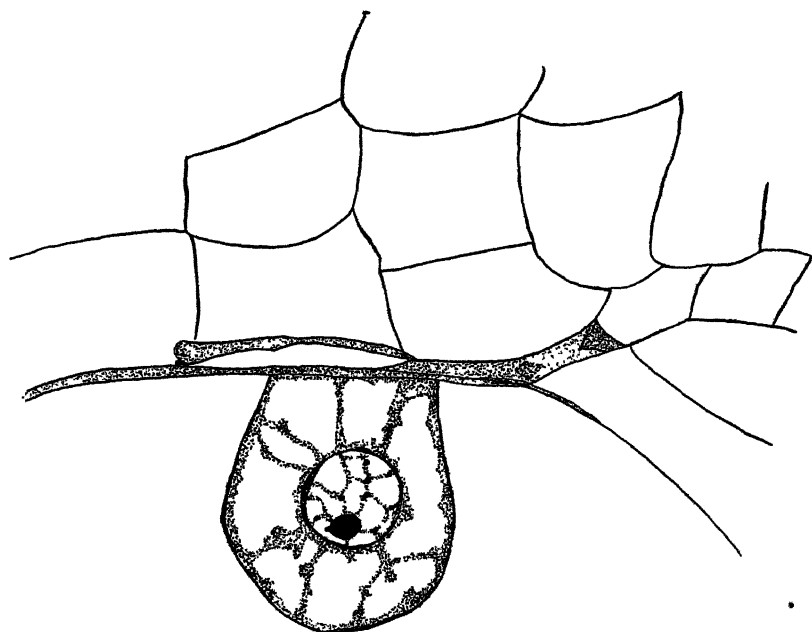
FIG. 3.—A 1 cell embryo. × 1095

VEGETATIVE STRUCTURES

The growth of the vegetative structures is gradual and continuous for about four months after pollination. The hull, shell, packing tissue, and middle septum of the nut in the early stages are similar to these respective regions, as previously described for the flower by the writers.

HULL

The hull is the modified calyx which is shed at maturity of the nut. The vascular system, sutures, color, and shape of the hull are similar from the time of pollination until the nut reaches full size in early September. An increased irregularity in the size and shape of the cells, increased evidence of tannin formation, an an increase in size and thickness of the entire region occurs during development (fig. 4h).

All varieties studied are divided into two natural groups on the basis of the prominence of the sutures. The thickness of the hull of varieties in group 1 is approximately the same at the sutures as midway between the sutures. The thickness of the hull of varieties in group 2 is 36.8 per cent greater at the sutures than midway between them (fig. 5). The various varieties are grouped as shown in Table 2.

TABLE 2.—*Thickness of hulls at sutures and between sutures, of varieties of pecans from group 1 and group 2*

Variety	Thickness of hull in millimeters—		Variety	Thickness of hull in millimeters—	
	At sutures	Between sutures		At sutures	Between sutures
Group 1			*Group 2—Continued*		
Mobile	4.34	4.34	Jerome	5.03	4.76
Nelson	4.34	4.34	Mantura	4.34	3.17
San Saba	2.12	1.90	Moneymaker	5 40	3.49
Teche	4.12	4.12	Moore	5.40	3.49
			Pabst	6.66	4.34
Average	3 73	3.68	Randal	5.08	3.17
			Russell	4.12	3.49
Group 2			Rome	6.35	4.12
Alley	4.12	2.85	Schley	5.03	4.12
Appomattox	4.76	3.81	Stuart	6.35	4.76
Atlanta	5.71	3.49	Success	3.81	2.54
Beverage	4.12	3.17	Unknown	5.08	3.49
Bradley	5.40	4.12	Van Deman	4.76	3.81
Centennial	6.35	4.76			
Frotscher	5.71	4.76	Average	5.17	3.78

SHELL

The shell is the ovarian wall, located directly beneath the hull; it becomes very hard just prior to maturity and is exposed after the shedding of the hull (fig. 4, s). The shell is incompletely divided by the sutures into two equal segments. At a depth of about four layers of cells beneath the surface a vascular bundle extends from the base to the apex of the shell along each suture (fig. 4, su'). The vascular bundles which separate the hull from the shell become separated from both regions at maturity.

The color, shape, vascular system, and cell structure of the shell are similar from the time of pollination until the beginning of shell hardening, about four and one-half months later. During the period of growth the entire region increases in size and thickness, but no further growth occurs after shell hardening is well begun. The hardening begins at the apex and progresses toward the base, the regions immediately surrounding the sutures hardening in advance.
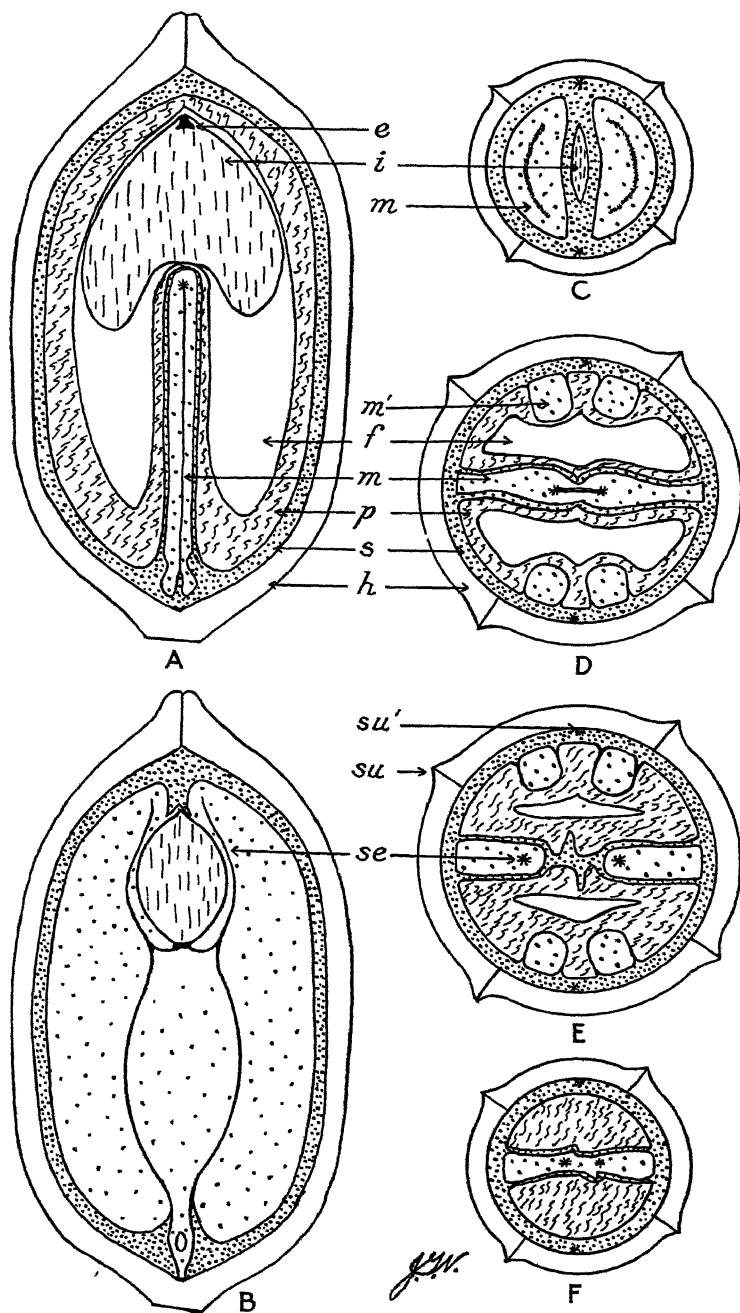
FIG. 4.—Location of the regions of the pecan nut: A, longitudinal section at right angles to the plane of the middle septum; B, longitudinal section in the plane of the middle septum; C, cross section just below the apex of the integument; D, cross section just below the opening in the middle septum; E, cross section of portion about three-fourths of the distance down from the apex; F, cross section through the lower region of the cavity of the shell; e, embryo; i, integument; m, middle septum; m', extension of the middle septum along the inner wall of the shell; f, fissure; p, packing tissue; s, shell; h, hull; su, suture of the hull; su', suture of the shell; se, fibrovascular bundles of the middle septum.

Approximately three weeks are required for the process to progress from the apex to the base.

MIDDLE SEPTUM

The middle septum, with the exception of an ovoid opening near the apex, divides the ovarian cavity into two parts. At maturity it forms an indelible partition between the two halves (cotyledons) of the kernel.

The septum is composed of two types of tissue, one being partially inclosed by the other. The inner is composed of large cells, and is soft and spongy during the growing period, but crumbly, reddish-brown in color, and bitter in taste in the mature nut (fig. 4, *m*). It extends irregularly from the base to the apex of the septum, enlarging at the apex to from the cap-like structure and extending baseward along the inner walls of the shell opposite to the attachment of the septum (fig. 4, *m'*). The second type of tissue of which the septum is composed is similar in cell structure to that of the shell. Beginning at the base, it extends to the apex of the
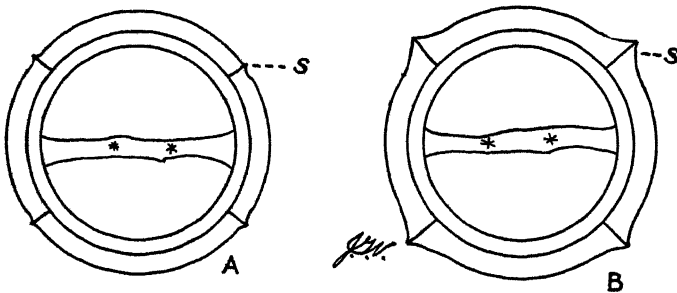
FIG. 5.—A, cross section of pecan hull from group 1, which, as is characteristic of the group, is approximately the same thickness at all points; B, cross section of a hull from group 2, which, as is characteristic of this group, is thicker at the sutures than midway between the sutures

embryo and forms a thin protective covering for the more delicate inner tissue of the septum.

The vascular bundles of the middle septum are embedded in the inner tissue (fig. 4, *se*). They enter through the base of the shell at two points. After entering the nut they curve outward from one another to come slightly inward again near the base of the opening in the septum. At this point a branch from each converges and enters the seed coat at the base of the opening, forming a complex system of vascular bundles. The point of entrance of the bundles of the septum into the seed coat is marked at maturity by an elliptical, slightly raised scar. The main bundles curve around the opening and continue toward the apex of the shell, terminating just before they reach the upper limits of the tissue in which they are embedded.

PACKING TISSUE

The remaining space within the young nut is filled with a white, dry, soft, spongy tissue that is present at the time of pollination but does not become distinct until several weeks later. It fills all space within the shell that is not occupied by the middle septum and has

the outline of the kernel of a completely filled nut (fig. 4, *p*). The crevice or fissure, mentioned by the writers in the description of the flower, becomes wider and deeper as the nut grows, so that a cavity or pocket is formed in the packing tissue on either side of the septum to accommodate the expanding seed coat (fig. 4, *f*). As the seed coat enlarges and fills the shell, the packing tissue is pressed closely against the walls of the shell and middle septum and at maturity forms a layer of dry, brittle material.

The packing tissue does not have a vascular system and is in a state of constant change, due to the growth and filling of the seed coat by the embryo. The cells bordering on the fissure are smaller than those composing the main body of the tissue.

If the embryo, and consequently the seed coat, does not completely fill the cavities of the shell, the packing tissue will not be pressed firmly against the walls; in which case a part of the tissue will cling to the seed coat at maturity as dry, brown, spongy fragments. Such nuts are popularly known as "chaffy" nuts.

REPRODUCTIVE STRUCTURES

INTEGUMENT OR SEED COAT

The ovule is about two-thirds inclosed by the integument at pollination time and is completely inclosed 10 to 15 days later. Growth and expansion is slow during the following two and one-half months. About July 15 the rate of growth of the integument, or capsule, increases. It expands into the fissures of the packing tissue (fig. 4, *i*), forces the latter against the surrounding walls, and reaches full size a month later. On becoming fully grown the integument becomes the seed coat, incloses the growing embryo, and soon assumes a definite and permanent shape (fig. 6, *a* and *b*).

The irregularities in the outline of the seed coat at maturity are due to the four projections from the caplike structure along the walls of the shell (described under middle septum) and to the unevenness in the surfaces of the middle septum. The seed coat of well-filled varieties, such as the Schley and Curtis, have a smoother surface at maturity than varieties not so well filled, such as the Frotscher and Teche.

An elliptical scar corresponding to that found at the base of the opening of the middle septum is present on the seed coat at the point of its attachment to the middle septum. Radiating from this scar are numerous vascular bundles which branch profusely and extend throughout the seed coat. The color of these bundles both before and after maturity is similar to that of the seed coat.

EMBRYO DEVELOPMENT OR "FILLING"

The nucellus, with the small embryo sac, fills the integument during the early stages of development. Active cell division has been observed during the first few weeks after pollination, but how long it continues was not determined. However, the nucellus is later absorbed as food material for the developing embryo. The embryo sac reaches a rather large size before the embryo begins growth.
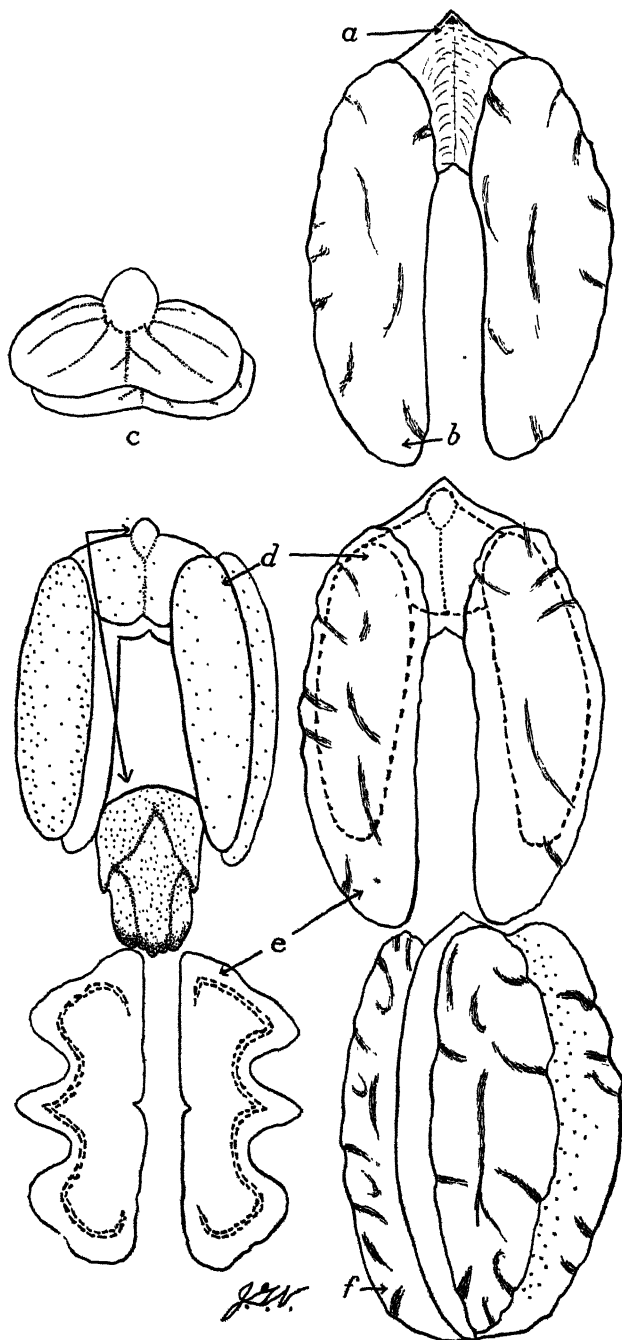
FIG. 6.—Manner of filling of the pecan nut: *a*, a young embryo about the second week in August; *b*, the seed coat, which was fully developed when filling began; *c*, the embryo about the last week in August; *d* and *e*, the rapidly developing cotyledons within the seed coat; *f*, the fully developed embryo about October 10

The seed coat enlarges much more rapidly than the embryo, the additional space being filled by a semiclear fluid.

During the earlier part of the development period considerable pressure is exerted by this fluid, thus forcing the seed coat outward and downward into all available space within the shell. (Fig. 4, *i.*) The pressure becomes less after the embryo fills about half of the interior of the seed coat.

Beginning with the 64 cell embryo (fig. 6, *a, b, c, d, e,* and *f*), previously mentioned as being present 12 weeks after pollination rapid growth follows for about five weeks. Two double winglike appendages (the cotyledons) develop on each side of the young embryo (fig. 6, *c*), growing sideways until the edges reach the walls of the seed coat, which causes them to turn toward the base. Expansion is first in length and width, then in thickness (fig. 6). Included within the folds of each cotyledon is a thin white membrane containing numerous vascular bundles. Previous to the thickening of the cotyledons this membrane is a thick succulent pad of material. The origin and purpose of this membrane has not been determined (fig. 7, *b*). Table 3 shows the varieties of pecans used in this work, as well as the relative stages of development of the seed coat, shell hardness, embryo development, and maturity, with the dates on which these occur.

TABLE 3.—*Seed-coat development, shell hardness, and embryo development at different periods, and dates of maturity, for different varieties of pecans*

Variety	Condition on Aug. 13 of—			Condition on Sept. 4 of—						Date of maturity in—	
	Seed coat development, 1926	Shell hardness, 1926	Embryo development, 1926	Seed coat development			Shell hardness		Embryo development, 1926		
				1924	1925	1926	1925	1926		1924	1926
	Per cent			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
Alley	20	Not hard	Not visible	15	100	100	5	50	5	Nov. 11	
Appomattox	15	...do	...do	15	50	100	30	60	20	Nov. 15	Nov. 6
Atlanta	15	...do	...do	60	15	100	5	30	0	Nov. 10	
Beverage	50	...do	...do	100	50	100	40	100	20	Oct. 15	Nov. 10
Bradley	10	...do	...do	10	30	90	5	15	0	Nov. 15	
Centennial	90	10 per cent	...do	100	70	100	90	100	100	Oct. 10	Oct. 17
Curtis	30	Not hard	...do	10	50	100	5	30	15	Nov. 10	
Delmas	----						5	10			
Frotscher	25	Not hard	Not visible	10	----	100	----	10	0	Oct. 25	Oct. 30
Indiana	90	50 per cent	...do	----	50	100	90	100	100	Oct. 5	
Jerome	90	40 per cent	...do	100	90	100	70	100	90	Oct. 10	Oct. 4
Mantura	65	Not hard	...do	100	70	100	30	100	30	Oct. 15	Oct. 17
Mobile	30	...do	...do	60	50	80	60	10	0	Nov. 15	Nov. 6
Moneymaker	90	10 per cent	...do	100	70	100	90	100	50	Oct. 15	Oct. 4
Moore	80	5 per cent	...do	60	70	100	5	100	50	...do	Oct. 17
Nelson	30	Not hard	...do	15	----	100	----	100	15	Nov. 11	Oct. 30
Pabst	60	...do	...do	20	90	100	5	90	20	Oct. 28	Nov. 6
Randal	30	...do	...do	15	15	100	5	10	0	Nov. 10	Nov. 12
Rome	90	50 per cent	...do	100	90	100	90	100	90	Oct. 10	Oct. 4
Russell No. 3	90	5 per cent	...do	65	15	100	5	90	15	----	Nov. 10
Russell	----			15	15	100	5				
San Saba	60	5 per cent	Not visible	65	50	100	10	90	50	Oct. 20	Do.
Schley	60	...do		15	70	100	5	----		Oct. 15	Oct. 17
Stuart	60	...do	Not visible	100	35	100	5	100	90	Oct. 18	Oct. 29
Success	60	...do	...do	70	70	100	75	100	5	Oct. 15	Oct. 4
Teche	25	Not hard	...do	15	70	90	50	10	0	Nov. 11	Oct. 30
Unknown	60	5 per cent	...do	----	----	100	----	80	20	Oct. 20	
Van Deman	60	...do	...do	15	----	100	----	30	0	Oct. 15	
Waukenah	----						5	10			
Average	54.4	7.7	0	50	52.2	98.5	31.6	63.9	31	Oct. 25	Oct. 25

<center>THE PROCESS OF RIPENING</center>

The process of ripening includes those chemical and physical changes which take place within the nut from the time the embryo reaches full size until it is palatable.

<center>CHEMICAL CHANGES</center>

Nine samples were taken for chemical analyses. The first seven were taken on successive weeks, beginning just before the seed coat was fully filled; two weeks elapsed between the time of taking the seventh and eighth sample, and five weeks between the time of taking the eighth and ninth. The nuts were ready for harvest at the time of the taking of the eighth sample. The Bradley and Mobile varieties, both of which mature very late in the season, were used. Fifty nuts constituted a sample. The nuts were hulled, shelled, dried at 70° C., and analyzed according to the methods used by the American Association of Official Agricultural Chemists (1). The results of the analyses are shown in Table 4.

There was a progressive change in the composition of the embryo until the time of taking the fourth sample, or four weeks before the time of harvest. However, after the fourth sample was taken the composition was fairly constant. At this time the color markings were faintly visible at the tip of the shell. Two weeks later the abscission of the shell and hull began and continued for two weeks, accompanied by the drying of the tissues of all the vegetative regions.

TABLE 4.—*Chemical changes which take place within the pecan nut during the growing period, as indicated by analyses*

<center>BRADLEY VARIETY</center>

Date of record	Weight of fresh sample in grams	Percentage of dry matter	Total weight of dry matter in grams	Ash		Protein (N×6.25)		Fiber		Nitrogen-free extract		Fat	
				Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams
Sept. 25	112.7	24.99	28.16	4.66	1.31	13.68	3.85	5.40	1.52	28.02	7.89	48.23	13.58
Oct. 4	143.5	29.01	41.63	2.82	1.17	12.03	5.01	5.72	2.38	19.03	7.92	60.40	25.14
Oct. 11[1]	166.0	38.01	63.10	2.95	1.86	15.66	9.88	5.12	3.23	16.38	10.34	59.89	37.79
Oct. 18	146.0	62.46	91.20	2.03	1.85	11.20	10.21	3.17	2.89	16.17	14.75	67.43	61.50
Oct. 25	149.0	65.53	97.65	1.80	1.76	11.49	11.22	3.55	3.47	16.18	15.80	66.98	65.41
Nov. 1	162.0	69.43	112.48	1.74	1.96	11.23	12.63	3.86	4.34	15.95	17.94	67.22	75.61
Nov. 8	172.0	70.78	121.75	1.65	2.01	11.75	14.31	3.41	4.15	15.59	18.98	67.61	82.32
Nov. 22	------	------	------	1.66	------	11.49	------	2.82	------	16.28	------	67.76	------

<center>MOBILE VARIETY</center>

Date of record	Weight of fresh sample in grams	Percentage of dry matter	Total weight of dry matter in grams	Ash		Protein (N×6.25)		Fiber		Nitrogen-free extract		Fat	
				Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams
Sept. 25	101.4	20.29	20.58	4.49	0.92	17.15	3.53	4.55	0.94	23.48	4.83	50.33	10.36
Oct. 4	155.0	42.77	66.30	2.55	1.69	13.82	9.16	3.60	2.39	20.73	13.74	59.30	39.32
Oct. 11	99.5	55.42	55.14	2.07	1.14	12.62	6.96	3.60	1.99	15.07	8.31	66.64	36.75
Oct. 18	144.0	60.18	86.67	1.83	1.59	13.26	11.49	2.37	2.05	16.65	14.43	65.89	57.11
Oct. 25	170.0	61.47	104.50	1.81	1.89	13.09	13.68	2.95	3.08	15.57	16.27	66.58	69.58
Nov. 1	171.0	65.46	111.95	1.72	1.93	13.34	14.93	2.49	2.79	14.23	15.93	68.22	76.37
Nov. 8	180.0	64.91	116.85	1.79	2.09	13.51	15.79	2.84	3.32	13.85	16.18	68.01	79.47
Nov. 22	------	------	------	1.96	------	13.94	------	2.89	------	13.38	------	67.82	------

[1] Teche variety.

From the table it will be noted that the dry matter of the embryo increased from 28.16 grams to 121.75 grams, and from 20.58 grams to 116.85 grams, respectively, for the two varieties. The first four

weeks were marked by an increase in weight of dry matter and per-
centage of oil and a decrease in percentage of ash, fiber, nitrogen-free
extract, and protein.   During the period covered by the last five
analyses the weight of dry matter and the percentage of oil increased
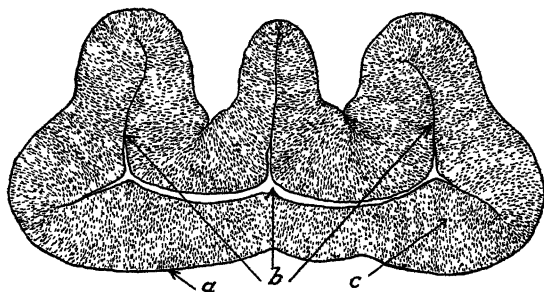
Fig. 7.—Cross section of pecan cotyledon: *a*, semipermeable
membrane inclosing the entire kernel; *b*, transparent membrane
in the center of the cotyledon; *c*, intermembrane tissue, practically
uniform in structure

slightly, while the ash, fiber, protein, and nitrogen-free extract
remained almost constant.   In total weight there was an increase in
ash, protein, fiber, nitrogen-free extract, and fat.

The decrease in the percentage of nitrogen-free extract is due to a
decrease in soluble carbohydrates.   The decrease in percentage of
fiber was probably due to a decrease in the ratio of the seed coat to
embryo as the seed coat was filling.   The increase in percentage of
oil is due to the transformation of carbohydrates into oil.
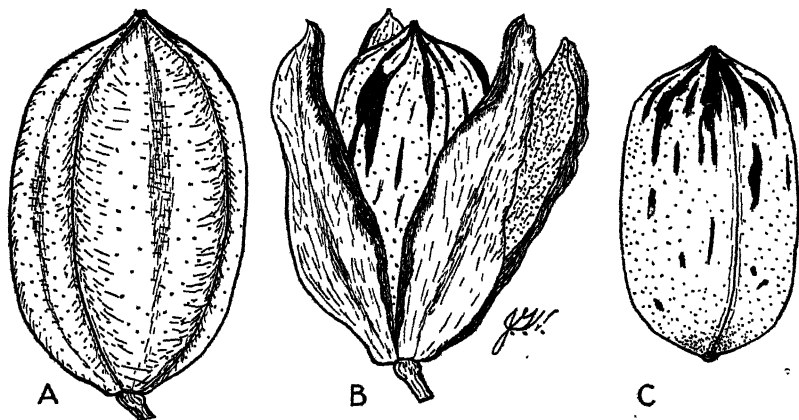
Fig. 8.—A, full-grown nut, Pabst variety, showing the sutures of the hull; B, hull opened along
sutures, and nut ready for harvest; C, nut showing color "markings"

MATURITY

Maturity may be said to begin as soon as all portions of the nut
reach full size, from five to six weeks before harvest.   All of the regions
begin to lose moisture and to change in color, beginning at the apical
end and progressing baseward.   Simultaneously with the ripening of

both hull and shell, as evidenced by the change in color, the hull divides into four nearly equal segments and separates from the shell, after which the nuts are ready for harvest (fig. 8.) The changes in color of the various regions of the nut during the process of ripening (4) are shown in Table 5:

TABLE 5.—*Color of the various regions of the pecan nut before maturity and when fully matured*

Region	Color before maturity	Color when fully mature
Hull	Pale green oxide of chromium	Greenish-black or cedar green.[1]
Shell	Pale gray green	Putty color.
Shell lining	Snow white	Dark fawn.
Packing tissue	do	Blood-red brown.
Seed coat	Amber white	Honey yellow.
Embryo	Snow white	Creamy white.

[1] If for some reason the hull and shell do not separate normally, the hull will be lighter in color at maturity.

Due to many causes, nuts do not mature normally. The presence of the shuck worm in the hull region, premature frosts, the mechanical severing of the nut from the tree, or other conditions which interfere with the function of any or all regions, will prevent the hull from dividing into segments and separating from the shell. The failure of the hull and shell to separate is an indication that the nut is partially or totally unfilled.

COLOR MARKINGS

The formation of the typical color markings on the shell of the pecan is a result of the process of separation of the hull from the shell. The vascular system between the hull region and the shell region becomes separated and is set almost free from both shell and hull. Normally, when the nut is removed this vascular material remains in the hull, but with care it may be removed with the shell. From two to five weeks before the nut is mature the middle lamellae of the cells of the hull surrounding the vascular bundles undergo changes which allow them to separate. The exact time or period when these cells are set aside as an abscission layer has not been determined, but examination of scrapings from the tissue about the bundles and from the color markings on the shell indicate that the color markings are the result of a deposit of the separated cells of the abscission layer. They appear as a single layer of large thin-walled cells.

The markings on the mature nut appear as dots or streaks. The streaks more commonly appear toward the apical end of the nuts, while the basal end has dots only. The relative number of dots and streaks on an individual nut is a varietal character. The Russell, Bradley, Curtis, Atlanta, and Centennial varieties have a large number of dots, while the Rome, Frotscher, and Alley varieties have numerous streaks and few dots. The markings may be removed from the shell by rubbing or brushing. The Frotscher, Van Deman, Mobile, and other varieties frequently lose a large part of the markings during the harvesting process.

Nuts from which the hulls were removed before ripening began failed to develop markings (fig. 9, B); nuts from which the hull was removed after the ripening process started but before it was com-
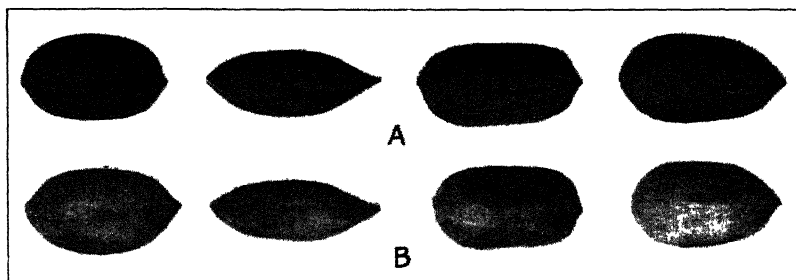
FIG. 9.—A, nuts with typical color markings; B, nuts without color markings as the result of girdling the shell a few weeks before maturity. Varieties, reading from left to right, Delmas, Atlanta, Pabst, and Pabst

pleted developed faint markings; and nuts from which one-half of the hull was removed before ripening, leaving the remainder intact, failed to develop markings where the shell was prematurely exposed.

FIG. 10.—Comparison of good and faulty nuts: Column 1, left to right, mature kernels in the shell; column 2, fully developed kernels; columns 3 to 10, inclusive, kernels showing various degrees of faultiness. Varieties by rows, reading from top to bottom, Schley, Pabst, Teche, and Mobile. The Mobile is the only variety that does not have fully developed seed coat in every case

FAULTY NUTS

Faulty nuts (fig. 10) are found in all varieties. The term is applied to nuts with seed coats partially or wholly unfilled at maturity. The term may also be applied to nuts that were normal at maturity but were later attacked by insects or fungi. •It is with those nuts

whose seed coats are partially or wholly unfilled at maturity that this study is most concerned because this condition is due to the activity within the nut itself, as contrasted with the condition of nuts attacked by insects or fungi, which are outside agencies.

Faultiness is correlated with other activities, either of the entire tree or some part of the tree, and may be traced to direct causes. In some cases it is traced to extremely dry weather which so interferes with the growth of the tree that it can not support nut development at the time the embryo is making rapid growth. Among prolific varieties, as the Mobile and Teche, a heavy crop may so tax the resources of the tree that filling of the nuts can not take place properly. Wide areas along the Gulf Coast produced faulty nuts in 1926 because of a storm which occurred September 20 defoliated the trees, and thus prevented photosynthetic activities. A very late variety, such as the Bradley or Randal, often produces a high percentage of faulty nuts as the result of early frost, which checks growth before filling is completed. Nuts were rendered faulty mechanically by girdling the hull region of varieties in various stages of embryo development. This has a direct result on the condition of the nuts at maturity. The effects of girdling on nuts are shown in Table 6.

TABLE 6.—*Effect of girdling on nuts, at various stages of their development*

Variety	Number of nuts treated	Condition of nuts when girdled	Condition of nuts at maturity
Rome	10	Shell hard; seed coat filled	All normally filled.
Mobile	20	Shell hard; seed coat 30 per cent filled	Poorly filled.
Atlanta	20	----do----	Do.
Teche	20	Shell hard; seed coat 20 per cent filled	Poorly filled; poor flavor.
Mobile	10	Shell partly hard; seed coat unfilled	Four partly filled; six unfilled.
Beverage	20	Shell partly hard; seed coat 5 per cent filled	Practically no filling.
Bradley	10	Shell partly hard; seed coat undeveloped	100 per cent drop.

Girdling nuts of the Rome variety after the seed coat was filled did not produce faulty nuts; girdling nuts of the Mobile, Atlanta, and Teche varieties which were partly filled produced faulty nuts; girdling nuts of the Bradley and Beverage varieties that were unfilled produced all faulty nuts. Faultiness has not been found to be related to the size or shape of the nut.

From a lot of nuts of the Pabst variety, those that had developed faint color markings were selected. The cracking strength of the shell and the filling was noted as is shown in Table 7.

TABLE 7.—*Relation of color markings and cracking strength of pecan nuts to faultiness, Pabst variety*

Condition of color markings	Number of faulty nuts	Number of good nuts	Pressure required to crack nuts (in pounds)
Very faint	109	4	61.0
Normal	6	110	68.2

Assuming these figures as typical of a large number of varieties, it may be stated that faintness of color markings is directly related to faultiness, and that low cracking strength is somewhat related to faultiness.

SUMMARY

Fertilization in *Hicoria pecan* Brit. occurs from 5 to 7 weeks after pollination, but the embryo does not become plainly visible until 10 weeks after pollination.

The pecan nut is made up of six distinct regions, viz, the hull, the shell, the middle septum, the packing tissue, the seed coat, and the embryo, the first four of which are purely vegetative.

The factors which influence the size of the nut operate during the early and middle part of the growing season, and those which influence "filling" operate during the latter part of the growing season.

Faulty nuts and undeveloped color markings are correlated, both conditions having been produced artificially.

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SULPHURIC ACID AS A WEED SPRAY [1]

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HISTORICAL REVIEW

EARLY WORK

One of the most striking methods of weed control is the use of selective sprays which will kill the weeds without injury, or with but slight injury, to the growing crop. These sprays are used for killing annual weeds in grain crops. When this method was first accidentally discovered, in 1896, by the French grape grower, L. Bonnet, it aroused great interest throughout the agricultural world, with the result that in the following years more or less extensive experiments were carried out.

In the United States Bolley (7),[3] who was the earliest worker to try out weed sprays, and he obtained excellent results. He states: "Farmers have been backward in applying what the writer believes to be one of the most effective methods for aiding the cereal producer in eradicating weeds." So successful were Bolley's experiments that he wrote: "The gain to the country at large * * * will be much greater in monetary consideration than that which has been afforded by any other single piece of investigation applied to field work in agriculture." However, after his experience in 1900 Bolley (6) changed his opinion to some extent. That year was dry, and it was shown that "these weeds [mustards, etc.] do not always die down by the treatment so easily, indeed that in dry slow-growing periods spraying should not be attempted." Bolley used several sprays, including soldium chloride, iron sulphate, copper sulphate, corrosive sublimate, and sodium arsenite.

Several other workers, Pammel and King (28), Moore and Stone, (23), Stone, (36), and others obtained similar results. The success of the spraying was connected rather closely with prevailing conditions for growth and weather during and after the spray had been applied. Schultz (34), Bornemann (8), Wehsarg (39), and other German authors described extensive experiments with various chemical sprays in Germany, where the method was utilized more than in any other part of the world. Extensive experiments are also reported from England and Scotland, since workers were very eager to try the new method of weed eradication. Brenchley (9) summarizes several of these experiments.

Many countries now possess a rather voluminous literature dealing with weed sprays. Sulphate of iron was the chemical used chiefly

Journal of Agricultural Research,
Washington, D. C.

Vol. 34, No. 11
June 1, 1927
Key No. N. Y. (Cornell) 10

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or exclusively. The results obtained are various and often contradictory. Under favorable conditions the results were excellent, but failures caused by rains or other complications are often reported.

A NEW PERIOD

In the earlier experiments various chemicals, usually solutions of salts, were used as sprays. Acids do not seem to have been tried to any great extent, and when used the results seem to have been no more satisfactory than with other sprays. For instance, Woods (*41*, *p. 100–101*) in 1904, reports that a 20 per cent solution of iron sulphate reinforced with 5 per cent sulphuric acid failed to destroy *Raphanus raphanistrum*. Experience indicates that *R. raphanistrum* is very easily killed by either sulphuric acid or iron sulphate. It is possible that Woods was working with another similar plant, possibly *Brassica campestris*.

However, in 1911 Rabaté (*29*) reports some results from experiments on weed eradication in winter wheat, which mark the beginning of a new period. He used solutions of copper sulphate, iron sulphate, and sulphuric acid. He concludes that sulphuric acid in 6, 8, or 10 per cent solutions, the degree of strength depending upon local conditions, is the most satisfactory spray to use. The solution was applied at a rate of 1,000 liters per hectare (107 gallons per acre). It killed most annual weeds but did not retard the growth of the wheat, although the lower leaves were killed. The sulphuric acid solution also had a fertilizing effect on the soil. Later on Rabaté (*30*, *31*), as well as other workers in France, reported similar results. Jaguenaud (*18*) found that sulphuric acid killed wild radish, wild mustard, crowfoot, vetches, and vetchling without injury to wheat. He used 7 liters of sulphuric acid (66° Baumé) to every hectoliter of water, which gave approximately a 10 per cent solution. Several papers deal with the eradication of weeds in flax (*17*, *19*, *32*). Morettini (*24*) in 1914–15 reports similar results in Italy. In sprayed fields he obtained an increase of 6 bushels of grain per acre.

In the Scandinavian countries, especially in Norway, the new method of weed eradication is now extensively used. Korsmo (*20*, *21*, *22*) started very comprehensive experiments on weed eradication in 1914. The chief spray he used was a diluted sulphuric acid. In spring-sown grain he found a strength of 3.5 to 4 per cent solution applied at a rate of 1,000 liters per hectare (107 gallons per acre), sufficient to kill all annual weeds to which the spray could adhere. As a result he obtained a very marked increase in yield. For instance, for the average of 211 experiments carried out from 1914 to 1922 in spring-sown grain crops, he obtained an increase of 490 kgm. of grain (25.3 per cent above unsprayed plots) per hectare. Calculated per acre the increase in yield of grain would be about 430 pounds.

Unfortunately, the results are published only in Norwegian, so that they are almost unknown outside of Scandinavia. In 1921 the writer (*1*, *2*, *4*) began experiments on weed eradication in Sweden under conditions similar to those of Korsmo, and has obtained very satisfactory results.

WEEDS REPORTED KILLED BY SULPHURIC ACID

Table 1, listing weeds reported killed by sulphuric acid, is compield from the reports of several workers.

TABLE 1.—*Weeds reported killed by sulphuric acid solution of different concentrations*

Name of weed	Investigator reporting	Percentage concentration of sulphuric acid solution used
Adonis aestiralis L. (summer adonis, or pheasant's-eye)	Morettini	10. 0
Anthemis arvensis L. (corn camomile)	Korsmo	3. 5 to 4. 0
Anthemis cotula L. (May weed or dog fennel)	____do	3. 5 to 4. 0
	Rabaté	10. 0
Brassica alba (L.) Boiss. (white mustard)	Korsmo	3. 5 to 4. 0
Brassica arvensis (L.) Ktze. (wild mustard or charlock)	Korsmo and others	3. 5 to 10. 0
Brassica campestris L. (rutabaga)	Korsmo	3. 5 to 4. 0
Capsella bursa-pastoris Med. (shepherd's purse)	____do	3. 5 to 4. 0
	Rabaté	10. 0
Centaurea cyanus L. (bachelor's button)	Korsmo	3. 5 to 4. 0
Chenopodium album L. (pigweed, lamb's quarters)	Aslander	3. 5 t0 4. 0
Chenopodium rubrum L. (red goosefoot)	Korsmo	3. 5 to 4. 0
Chenopodium polyspermum L. (pigweed or lamb's quarters)	____do	3. 5 to 4. 0
Chrysanthemum segetum L. (yellow oxeye)	____do	3. 5 to 4. 0
Cuscuta sp. (dodder)	Rosa (*33*)	4. 0 to 5. 0
Daucus carota L. (wild carrot)	Morettini	10. 0
Delphinium consolida L. (larkspur)	Korsmo	3. 5 to 4. 0
Erophila verna E. Mey	Rabaté	10. 0
Erysimum cheriranthoides L (wormseed mustard)	Korsmo	3. 5 to 4. 0
Euphorbia peplus L. (petty spurge)	____do	4. 0
Fagopyrum tartaricus Gaertn. (Tartarian buckwheat)	____do	3. 5 to 4. 0
Galeopsis tetrahit L. (hemp nettle)	____do	3. 5 to 4. 0
Galeopsis speciosa Mill	____do	3. 5 to 4. 0
Galium aparine L. (cleavers)	____do	3. 5 to 4. 0
Lamium amplexicaule L. (henbit)	____do	3. 5 to 4. 0
Lamium purpureum L. (dead nettle)	____do	3. 5 to 4. 0
Lapsana communis L. (nipplewort)	____do	3. 5 to 4. 0
Lathyrus spp. (wild peas)	Jaguenaud	10. 0
Lepidium campestre (L.) R. Br. (field peppergrass)	Korsmo	3. 5 to 4. 0
Lepidium ruderale L. (peppergrass)	____do	3. 5 to 4. 0
Matricaria discoidea DC. (pineappleweed)	____do	3. 5 to 4. 0
Matricaria chamomilla L. (wild chamomile)	Jaguenaud	10. 0
Papaver argemone L	Korsmo	3. 5 to 4. 0
Papaver dubium L. (poppy)	____do	3. 5 to 4. 0
Papaver rhoeas L. (field poppy)	____do	3. 5 to 4. 0
	Rabaté	10. 0
Polygonum aviculare L. (knotweed, or knotgrass)	Korsmo	3. 5 to 4. 0
Polygonum convolvulus L. (black bindweed, or wild buckwheat)	____do	3. 5 to 4. 0
Polygonum lapathifolium Ait. (smartweed)	____do	3. 5 to 4. 0
Pteris aquilina L. (bracken, or brake fern)	Gordon (*14*)	5. 0
Ranunculus arvensis L. (buttercup)	Rabaté	10. 0
	Morettini (*25*)	10. 0
Ranunculus ficaria L. (lesser celandine)	Rabaté	10. 0
Ranunculus spp. (buttercups)	Jaguenaud	10. 0
Raphanus raphanistrum L. (wild radish)	Korsmo	3. 5 to 4. 0
Senecio vulgaris L. (groundsel)	____do	3. 5 to 4. 0
Sisymbrium officinale (L.) Scop. (hedge mustard)	____do	3. 5 to 4. 0
Sisymbrium sophia L. (flixweed)	____do	3. 5 to 4. 0
Solanum nigrum L. (black nightshade)	____do	3. 5 to 4. 0
Sonchus oleraceus L. (sow thistle)	____do	3. 5 to 4. 0
Specularia speculum Alph. DC. (Venus's-looking-glass)	Morettini	10. 0
Spergula arvensis L. (corn spurry)	Korsmo	3. 5 to 4. 0
Stellaria media (L.) Cyrill. (chickweed)	____do	3. 5 to 4. 0
Thlaspi arvense L. (penny cress, French weed)	____do	3. 5 to 4. 0
Urtica urens L. (nettle)	____do	3. 5 to 4. 0
Vicia spp. (vetches)	Jaguenaud	•10. 0
Viola tricolor (hearts ease)	Korsmo	3. 5 to 4. 0

Table 1 shows a wide range of concentration of sulphuric acid used. Korsmo never used more than a 4 per cent solution and obtained excellent results. In France and Italy a 10 per cent solution is commonly claimed to be necessary for the complete destruction of the weeds. This difference probably is due chiefly to the fact that Korsmo has been working in spring-sown crops, while the later data are obtained in fields of winter wheat sprayed in February or March. Rapidly growing plants in spring-sown crops are much more sus-

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ceptible than winter annuals in wheat. This point will be fully discussed later. This apparent difference in the susceptibility of plants may also be due in part to the different types of spraying machines employed. Korsmo had a machine constructed especially for spraying sulphuric acid, which worked very satisfactorily. The possibility of killing a certain weed by a dilute sulphuric-acid solution depends on the amount of spray actually adhering to the plant, which in turn depends to a great extent upon the type of spraying machine used. The writer's experiments on weed eradication have confirmed the importance of a good spraying machine in obtaining good results. That the importance of the proper spraying machine has sometimes been overlooked is clear when a writer recommending a 10 per cent solution declares that the spray can be distributed by a watering can.

WEEDS REPORTED BADLY INJURED BY SPRAY OF SULPHURIC ACID

Certain weeds are not killed by sulphuric-acid spray. Among these are many perennial weeds the leaves of which may be destroyed while the roots are unharmed, thus allowing new shoots to appear soon. Spraying of growing crops is not directed against perennial weeds but against annual, winter annual, and possible biennial weeds. Table 2, which lists some of the weeds reported badly injured by sulphuric-acid spray does not therefore, embrace all weeds known to be harmed. This does not mean that sprays, especially sulphuric-acid sprays, may not be of some importance in combating some perennial weeds. For instance, it is the writer's experience that spraying an oat field at the proper time will prevent shoots of *Cirsium arvense* (Canada thistle) from flowering. Dehn (*13*) states that the best method of eradicating weeds in lawns is to apply some drops of sulphuric acid on the crown of each plant.

TABLE 2.—*Weeds reported badly injured by spraying with sulphuric-acid solution*

Name of weed	Investigator reporting	Percentage concentration of sulphuric acid solution used
Adonis flammea Jacq	Rabaté	10
Agrostemma githago L. (corn cockle)	do	10
Centaurea cyanus L. (bachelor's button)	do	10
Lathyrus aphaca L. (wild pea)	do	10
Lathyrus hirsuta L. (wild pea)	do	10
Scandix pecten-veneris L.	do	10
Vicis angustifolia Reichard (vetch)	do	10
Vicia cracca L. (wild vetch)	do	10

WEEDS NOT INJURED BY SPRAYS OF SULPHURIC ACID

Several weeds are reported as not injured by sulphuric acid. A waxy surface and the concealing of the easily injured growing point protects the grasslike weeds as well as the grain plants. Dense hairs or glandular hairs protect some weeds against the sulphuric acid spray as well as against other sprays which act on the top of the plants. Several weeds have a smooth surface to which the spray can not adhere.

TABLE 3.—*Weeds reported not injured by spraying with sulphuric-acid solution*

Name of weed	Investigator reporting	Percentage concentration of sulphuric-acid solution used
Allium rotundum L. (wild onion)	Morettini	10 0
Allium riniale L (wild garlic)	{----do---- / Voelcker (37)	} 10. 0
Alopecurus agrestis L. (foxtail grass)	Rabaté	10. 0
Anchusa officinalis L. (alkanet)	Korsmo	3 5 to 4 0
Avena fatua L (wild oats)	Several workers	3. 5 to 10. 0
Avena strigosa Schreb	Korsmo	3. 5 to 4 0
Bromus mollis L	----do	3. 5 to 4 0
Bromus secalinus L. (chess or cheat)	----do	3. 5 to 4 0
Carduus crispus L	----do	3. 5 to 4 0
Cirsium lanceolatum Scop (bull thistle)	----do	3. 5 to 4 0
Erodium cicutanium L'Her. (stork's-bill)	----do	3. 5 to 4 0
Euphorbia helioscopia L. (wartweed)	----do	3. 5 to 4 0
Fumaria officinalis L. (fumitory)	----do	3. 5 to 4 0
Lolium temulentum L. (darnel)	----do	3. 5 to 4 0
Matricaria inodora L	----do	3. 5 to 4. 0
Medicago spp.	Morettini	10. 0
Muscari spp. (grape hyacinth)	----do	10. 0
Ornithogalum spp	----do	10. 0
Senecio viscosus L	Korsmo	3 5 to 4 0
Sonchus asper L., Hill	----do	3 5 to 4 0

Tables 1 to 3, inclusive, show that most weeds, not perennial, are killed when sprayed with sulphuric acid. Those unharmed are comparatively few and generally not so troublesome. These tables are not intended, however, to give the impression that sulphuric acid is the only spray which will kill these weeds. Under favorable conditions a solution of iron sulphate or copper sulphate may produce just as good results. Reports of experiments show, nevertheless, that the latter sprays often fail. Some of the reasons for this failure will be considered later in this paper.

PREVIOUS EXPERIMENTAL WORK

In hundreds of field trials it has been demonstrated that dilute sulphuric acid is an excellent spray. However, the action of the acid on plant tissues seems not to have been studied to any great extent. Recently the writer (3) has been able to show how sulphuric acts upon plants. Since a knowledge of this action is necessary for a further discussion, a brief summary is given here.

Several weeds and crop plants, grown in crocks in the greenhouse, as well as *Brassica arvensis* (mustard) growing in the field, were sprayed with diluted sulphuric acid solutions of various strengths. Because they were so hardy that they were not harmed by weak sprays the action of the sprays on mustard plants growing in the field was very easily studied. Cross sections of the sprayed and unsprayed leaves were made by the paraffin method. These sections were then examined microscopically to determine the action of the sulphuric acid upon the tissues of the leaves. The most striking action of the sulphuric-acid spray was as follows:

(1) After adhering to the surface of the plants it penetrates very rapidly and kills the protoplasm almost instantly. Leaves of mus-

tard have stomata on both sides (fig. 1, A). It was found that the acid at first penetrates through the stomata, destruction of the cells being first noticeable in the neighborhood of these openings; but it
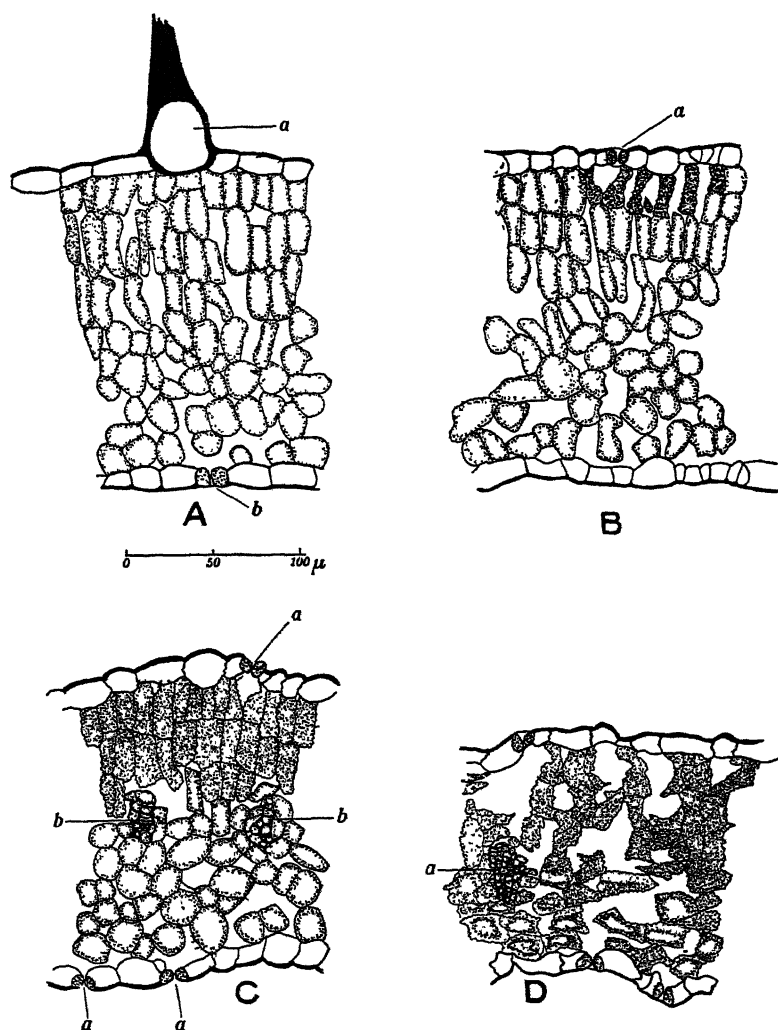
FIG. 1.—The action of sulphuric acid on plant tissues: (5) A, section of leaf of *Brassica arvensis* plant grown in the field during the winter (in England): *a*, hair on upper epidermis; *b*, stoma. B, section of leaf of field-grown plant one hour after it was sprayed with a 3 per cent solution of sulphuric acid: *a*, stoma through which the acid has penetrated and killed some cells. C, leaf similar to B, but showing the effect of the acid three hours after spraying: *a*, stoma; *b*, vascular bundles, diagrammatic. The acid has killed the upper half of the leaf, but the amount of the spray applied was not sufficient to kill the whole leaf. D, section of leaf three hours after it was sprayed with a 5 per cent solution of sulphuric acid: *a*, vascular bundle, diagrammatic. The whole leaf has collapsed as the amount of spray exceeded the quantity which the leaf was able to withstand. Note that the epidermis is the least injured part of the leaf

also seems to penetrate through the epidermal cells, as destruction was soon noticed in cells between the stomata. Figures 1 to 4 show the gradual increase of destruction caused by the penetrating acid.

(2) The sulphuric acid spray decomposes the chlorophyll as it unites with the magnesium atom of the chlorophyll molecule. (Fig. 1, B.) In order to prove this action of the acid, a few drops of 1 per cent solution of sulphuric acid were added to an alcoholic extract of chlorophyll from nettle leaves. It was found that four drops of this acid to five c. c. of chlorophyll extract immediately changed the chlorophyll color from deep green to yellowish green. This change in color indicates that the chlorophyll was split into phaeophytin and magnesium sulphate. According to Willstätter (*40*) this reaction may be indicated by the formula:

$$C_{55}H_{72}O_5N_4Mg + H_2SO_4 = C_{55}H_{72}O_5N_4H_2 + MgSO_4, \text{ or}$$
Chlorophyll + sulphuric acid = phaeophytin + magnesium sulphate
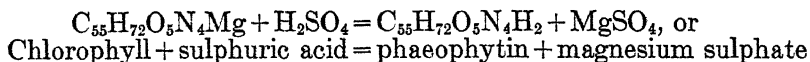
Only a very small amount of highly diluted acid is necessary to bring about this reaction, since the amount of chlorophyll in leaves is less than 1 per cent of the dry weight.

(3) It breaks up the chloroplasts. In sections of unsprayed leaves and in cells not yet affected by the acid the chloroplasts were plainly visible. In cells penetrated by the acid the cell contents formed a deeply stained mass in which no chloroplasts could be seen. (Fig. 1, C.) Apparently the acid breaks up the structure of the plastids.

(4) The acid does not destroy the cell walls, at least not those of the epidermis. Sulphuric acid is generally known to be very corrosive. It would seem then that it would "burn" the cell wall as it does clothes and other objects. But Figure 1, A to D, inclusive, shows that the epidermal cells are the least injured parts of the sprayed leaves. This may be explained by the fact that sulphuric acid of low concentration does not dissolve cellulose, which is regarded as the principal constituent of the cell walls. (Fig. 1, D.) The cell walls of the spongy parencyhma of sprayed leaves of pot-grown plants sometimes seemed to be destroyed by the spray. These delicate cell walls are supposed to be built up mostly of pectin (or pectin in combination with calcium) compounds which are dissolved by weak solution of sulphuric acid. However, the destruction of these leaves after the spray was applied was so rapid and complete that the fate of the cell walls was difficult to determine.

Further, it was found that plants grown in the greenhouse were easily destroyed by a 2 per cent solution while plants in the field which had been growing during the winter (in England) required no less than a 5 per cent solution to kill them. The same quantity of spray was used in all cases. As the acid can not evaporate, the plants must be able to absorb a certain amount without being harmed. Death follows spraying only when the sprayed quantity exceeds this amount.

Analyses of the plants showed that the field-grown plants had a far greater amount of dry matter, especially of ash, than the greenhouse-grown plants. This suggested that up to a certain point some of the constituents of the ash were able to neutralize the acid.

It was further found that the leaves of the greenhouse-grown plants changed in anatomical structure with their height above the cotyledons. They became more compact up to the fifth or sixth leaf, the number developed during the time of the investigations.

In reports on spraying experiments it is frequently stated that weeds are most difficult to kill when sprayed in the late rosette stage. If the leaves become more compact in anatomical structure from the cotyledons upward it perhaps would be possible to explain their resistance by changes in anatomical structure accompanied by an increased amount of ash and dry matter. Further work is needed, however, to clear up this point.

Observations clearly indicate that grain crops are unharmed by a spray of sulphuric acid. Their resistance is due to a cutin layer which prevents the sprays from adhering to the plants. The concealed growing point is an additional protection, as several workers have pointed out. In field experiments it is always observed that the ends of the leaves of the grain plants are "burned," the day after spraying has been performed. The lower parts of the leaves are generally unharmed. The writer (*3*) has found this due to the fact that the cutin layer on the lower surface of the leaves is less protective than that on the upper surface. When the leaves of the grain plants have reached a certain size the ends turn over, so that the lower surface comes uppermost. Hence, this part of the leaf is hit by the spray and destroyed. The larger the grain plants are when sprayed the greater is the killed portion of the leaf. In windy weather the sprays will adhere more easily to the lower surface of the leaves and injure the crop.

In some crock cultures (*3*) a 10 per cent solution was sprayed on oats and barley without causing more harm than the weaker solution, as only a very small amount adhered to the plants. It was further found that the amount of spray per unit of area had some influence on the injury. When the commonly used quantities were sprayed only a few leaves were harmed; if larger amounts were used more leaves were injured. The smaller the grain plants and the more vertical their growth the less was the injury.

Peas (*Pisum sativum*), and red clover (*Trifolium pratense*), are found to be uninjured by sprays of sulphuric acid. The writer (*3*) has pointed out that red clover is protected against the sulphuric acid by dense hairs on the leaves. However, the cotyledons are unprotected and therefore are injured. Field trials have shown that a spray of sulphuric acid in a grain field, in which clover seed has been sown, does not harm the clover plants provided they have developed some true leaves. The leaves of peas are rather waxy, so that the spray does not adhere to them.

In some water cultures where seedlings of barley and beans were placed in a full nutrient solution, to which were added increasing amounts of sulphuric acid, the writer (*3*) found a concentration of the acid of 1:20,000 did not injure the plants. Weaker solutions seemed to stimulate the growth. (See fig. 2.)

EXPERIMENTAL WORK

The experiments here reported were conducted to determine the influence of various environmental factors, and of the structure and composition of the plants, upon the effectiveness of sulphuric acid as compared with a solution of iron sulphate when used as weed sprays.

MATERIALS AND METHODS OF PROCEDURE

Plants of field mustard, *Brassica arvensis* (L.) Ktze., and Cornellian oats, *Avena sativa* L., were grown in glazed crocks filled with soil. When the plants had grown to the desired size, they were sprayed
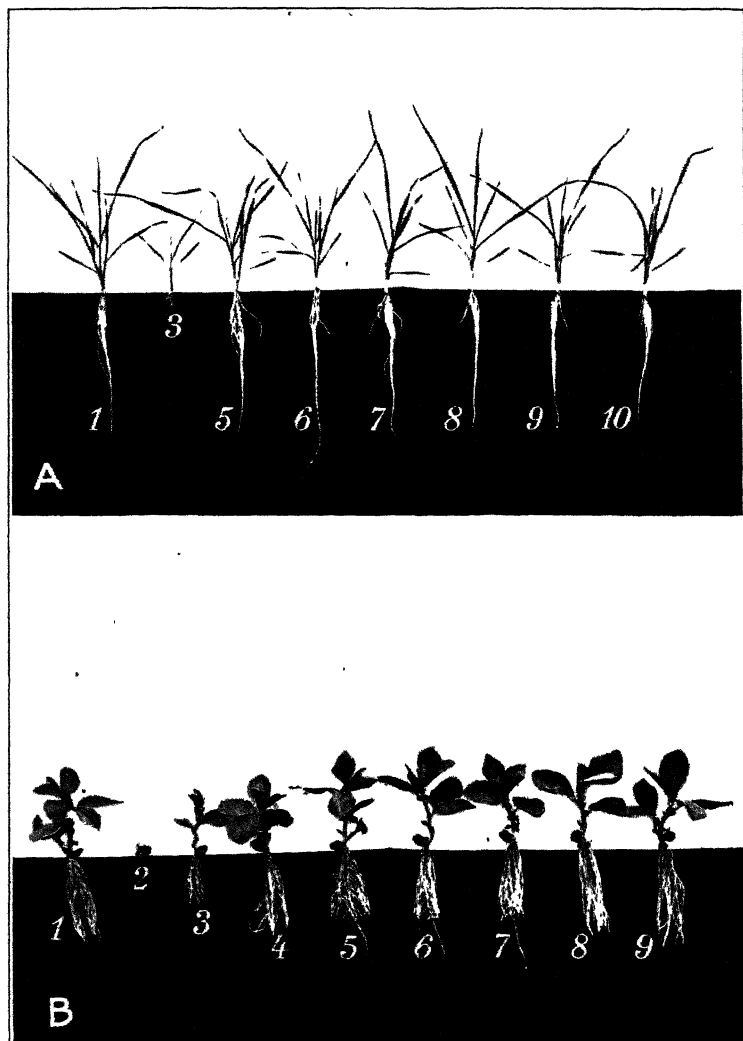
FIG. 2.—Effect of sulphuric acid on plant growth: A, Plants of barley grown in full nutrient solution to which were added increasing amounts of sulphuric acid; B, plants of beans (*Vicia faba*) grown in solutions similar to those in A
Amount of sulphuric acid in solution: 1, 0:1 (no acid); 2, 1:2,500; 3, 1:5,000; 4, 1:10,000; 5, 1:20,000; 6, 1:80,000; 7, 1:320,000; 8, 1:1,128,000; 9, 1:5,120,000; 10, 1:20,480,000
The concentration 1:20,000 had no injurious effect on the plants; weaker solutions seem to have stimulated the growth

with a solution of iron sulphate and dilute sulphuric acid and thereafter placed in glass chambers, where the atmosphere was kept at varying degrees of saturation. Observations were then made on the

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effect of the sprays on the plants. The leaves of mustard from some of the crocks were analyzed for dry matter and ash content. Samples of leaves were embedded in paraffin for study of structure.

In order to test the influence of the water content of the soil on the effect of the sprays, plants were grown under three conditions of soil moisture content. For this purpose glazed gallon crocks filled with sifted silt loam, into which had been mixed 10 per cent coarse sand, were used as culture vessels. The crocks held 4 kgm. of soil. The moisture content of the soil, at the time the crocks were filled, was found to be 8.3 per cent in the first experiment, and when the experiment was repeated 12.1 per cent.

In order to secure a uniform distribution of water throughout the soil, a special method was devised for watering the crocks (5). The water was applied through a small flower pot placed in the upper soil of the crock. From the flower pot the water was distributed laterally through four radiating "arms" of coarse sand which were inserted about 1 inch below the top of the soil. This method provided a very uniform distribution of soil moisture.

The seeds of mustard and oats were sown February 8, 1926. The plants in each crock were thinned to four oat plants and eight mustard plants. In the repeated experiment the plants were sown on March 22.

In order to obtain an even germination, all of the cultures were at first watered uniformly with a sprinkling can. This method was continued until the cotyledons were well developed. After this the cultures were divided into three lots and the water content was maintained at 15, 30, and 45 per cent of the water-holding capacity of the soil as determined by Hilgard's (16) method. Table 4 shows the moisture of the soil of the series in the two experiments.

TABLE 4.—*Moisture content maintained in soil in culture crocks containing mustard and oats*

Test series and experiment Nos.	Average original moisture content of soil	Average weight of water-free soil	Average water-holding capacity of soil	Moisture content of soil maintained during experiments	Quantity of water to be added to soil of original weight of 4 kgm.	Weight of soil plus moisture per crock
	Per cent	*Kgm.*	*Per cent*	*Per cent* [a]	*Gm.*	*Kgm.*
Test series No. 1:						
Experiment 1				15	70	4.070
Experiment 2				15	[b] −10	3.990
Test series No. 2:						
Experiment 1				30	460	4.460
Experiment 2				30	460	4.460
Test series No. 3:						
Experiment 1	8.3	3.668	41.9	45	860	4.860
Experiment 2	12.1	3.516	.47.2	45	930	4.930

[a] Indicated as percentage of water-holding capacity.
[b] Crock allowed to evaporate 10 gms. of water in order to come down to 15 per cent moisture.

The water content of the crocks was maintained at a uniform stage by keeping the weight of the crocks constant. As long as the plants were small, so that the water content changed but slowly, the crocks were weighed every second or third day depending on the temperature; but as the plants grew larger it was necessary to water them every day, especially those in medium moist and wet soil.

The average quantities of water lost by the different cultures varied. In experiment No. 1 of series 1, 2, and 3, for the period February 22 to March 14, the losses were 370, 950, and 870 gm., respectively. In experiment No. 2 the temperature was higher, so that the loss of water was greater. During the period April 4 to 22 the loss for experiment No. 2 in series 1, 2, and 3, was 380, 1,260, and 1,170 gm., respectively. These losses represent evaporation from the surface of the soil as well as transpiration by the plants.

As the season gave rather insufficient sunlight for growing strong plants, four 60-watt electric lights were mounted over the cultures and turned on from sunset until midnight.

The temperature of the greenhouse was held at 10° C. during the night. During the daytime the temperature rose depending on the amount of sunlight. Ventilation often proved insufficient to keep the temperature below 16° to 18° C. As a result the plants grew very rapidly and became more succulent than if they had been grown in the open.

SPRAYS USED AND THEIR APPLICATION

The effect of a spray depends to a certain degree on its concentration. If its effect is to be studied the most reliable data may be obtained by the use of a solution of low concentration as differences are most easily observed with such a solution. For this reason, and as the plants were rather succulent, sulphuric acid in concentrations of 1, 1.5, and 2 per cent by weight was used in the present study. The iron-sulphate solutions used in experiment No. 1 were always five times stronger than the sulphuric-acid solutions with which they were to be compared, namely 5, 7.5, and 10 per cent. The writer's experience in field trials indicates that a 20 per cent solution of iron sulphate is about as effective a weed spray as a 4 per cent solution of sulphuric acid. In some of the later applications in experiment No. 2 the concentration of the iron sulphate was increased to 15 per cent.

The plants were sprayed when they had developed four leaves which quite generally is regarded as the best time for this operation. For applying the sprays two types of atomizers were used. Since the sprayers were mounted on graduated cylinders it was easy to determine the amount of spray used. As nearly as possible 1 gm. per square decimeter was applied, equivalent to 1,000 liters per hectare or 107 gallons per acre, which is the amount generally applied under field conditions. The sprayings were performed during the forenoon when the temperature of the greenhouse was about 16° to 18° C. Each culture to be sprayed was divided by a screen of cardboard into two equal parts. One side was sprayed with sulphuric acid and the other with iron sulphate solution.

In order to test the possible influence of the humidity of the air on the effect of the sprays, the sprayed plants were exposed to three different conditions of humidity: (1) to a moist chamber of 90 to 100 per cent relative humidity; (2) to a dry chamber of about 30 per cent relative humidity; (3) to a greenhouse room in which the relative humidity was kept at about 60 per cent. The chambers used in these experiments were the same as those described by Muenscher (*26*). The temperature of the chambers was kept low by shading. The humidity of the greenhouse room was regulated by sprinkling with water and by ventilation.

EFFECT OF SPRAYS ON MUSTARD PLANTS WHEN APPLIED UNDER VARIOUS CONDITIONS

From the experiments made in spraying plants with a solution of iron sulphate the following general deductions may be made:

(1) If the humidity of the atmosphere is low, so that evaporation is rapid, white crystals are soon formed on the surface of the leaves. The epidermis under the crystals may show a natural green color or it may be blackened, depending upon the hardiness of the leaves. The blackening of the leaves increases until they are completely black and dry. The petioles are generally not affected until the leaf is completely destroyed, as the spray seems not to adhere so readily to them. If the spray is strong enough, however, both petioles and stems will be killed.

(2) If the humidity of the air is high enough to prevent or greatly retard evaporation, crystals are not formed. The surface of the leaves becomes black under the drops of the spray, and the plants become flaccid. This continues until the leaves are dead.

Plants sprayed with sulphuric acid of sufficient strength react as follows:

(1) The plants soon become flaccid.

(2) At about the same time yellow spots appear under the drops of the spray or after the water of the spray has evaporated, so that the spray seems to have disappeared. These yellow spots soon turn brown and increase in size until the whole leaf is discolored. At the same time the leaf tissues begin to dry up. Generally the petioles and the stems of the plants are affected as soon as the leaves. The spray seems to adhere very easily to these parts of the plants. (See fig. 3.)

INFLUENCE OF SOIL MOISTURE ON THE EFFECT OF THE SPRAYS

Soil moisture affected the development of the plants and thus indirectly the effect of the sprays. In the soil with the lowest water content, 15 per cent of water-holding capacity, the plants grew slowly. The color of the plants was a deeper green than those in the other series. The leaves were smaller and the internodes were shorter than on plants grown in soil with higher moisture content, and the plants appeared sturdier and were more hairy. In soil with medium water content 30 per cent of the water-holding capacity, the plants grew largest. The color was light green and the plants appeared rather succulent. Plants grown in wet soil, 45 per cent of water-holding capacity, appeared very similar to those grown in the medium moist series, except that they were somewhat smaller.

This difference in habitat had a marked influence on the effect of the sprays. However, the effect of a spray of iron sulphate was closely dependent upon the prevailing relative humidity of the air. It will be described therefore when that factor is considered. The action of sulphuric acid was dependent upon the strength of the solution. A 1 per cent solution failed to kill the plants in any of the series. The leaves became more or less scorched. Scattered over the surface were smaller or larger spots of dead tissues. A 1.5 per cent solution killed the plants of the medium moist and wet soil, while plants grown in the dry soil required a 2 per cent solution to kill them. The amount of spray was always 1 gm. per square decimeter.

INFLUENCE OF THE RELATIVE HUMIDITY OF THE AIR ON THE EFFECT OF SPRAYS

As stated before, the plants were exposed to various conditions of atmospheric humidity after they had been sprayed. The influence of the humidity was rather marked.

INFLUENCE OF RELATIVE HUMIDITY ON THE EFFECT OF IRON SULPHATE SPRAY

In the moist chamber where no evaporation took place, the relative humidity of the air being maintained at or near 100 per cent, the
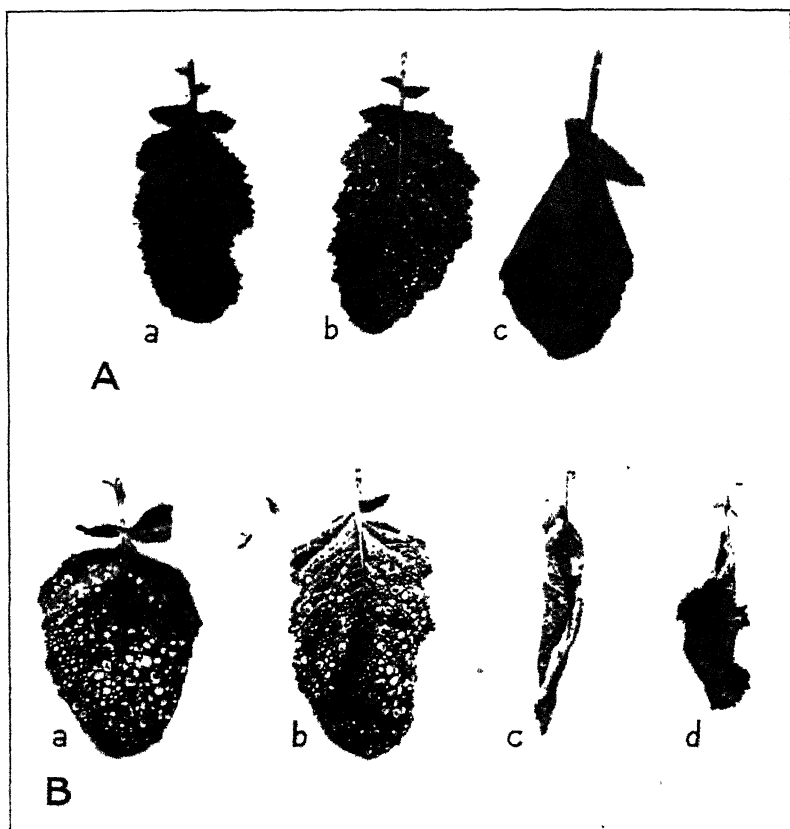
FIG. 3 —Effect of sprays on leaves of mustard: A, leaves one hour after spraying: *a*, unsprayed leaf; *b*, leaf sprayed with a 15 per cent solution of iron sulphate, on which crystals have formed, although the leaf is quite turgid; *c*, leaf sprayed with a 2 per cent solution of sulphuric acid, which was very flaccid and probably dead. B, leaves one day after spraying. *a* and *b*, leaves sprayed with iron sulphate, covered with crystals but still turgid; *c* and *d*, leaves sprayed with sulphuric acid which have dried and shriveled up

spray acted fairly rapidly. A blackening of the leaves under the drops of the spray was observed after four hours. The majority of the leaves were then more or less flaccid. After 24 hours the plants were completely destroyed. A 5 per cent solution proved strong enough to kill all mustard plants. (See fig. 4.)

With plants sprayed in the greenhouse, where the relative humidity was kept around 60 per cent, the effect of the spray was markedly different. As the spray evaporated, crystals appeared on the leaves.
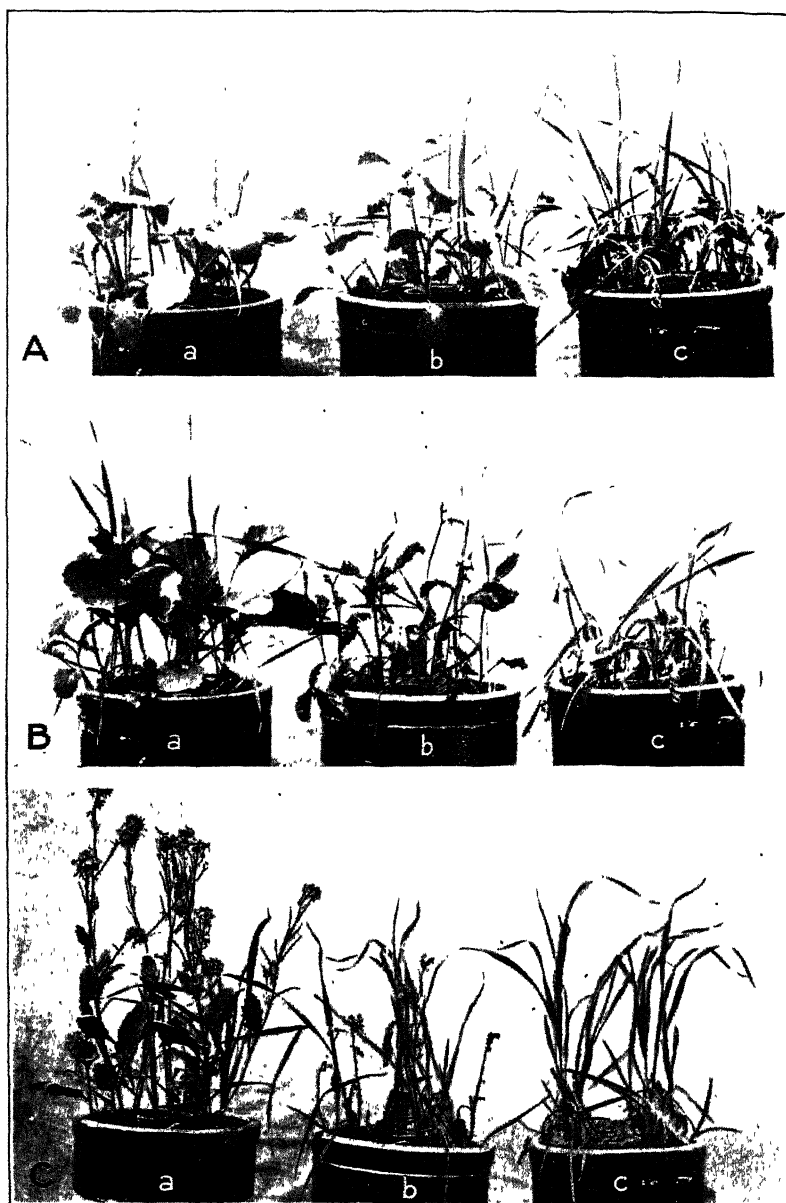
FIG. 4.—Influence of the relative humidity of the air on the effect of sprays. In each case (A, B, and C), a is the check crock containing plants which were unsprayed; b contains plants which were sprayed with a 15 per cent solution of iron sulphate; and c contains plants sprayed with a 2 per cent solution of sulphuric acid: A, appearance of plants one hour after spraying. Plants sprayed with sulphuric acid, crock c, are already dead, while the iron-sulphate spray has formed crystals on the leaves of the mustard plants without great damage to them. B, plants one day after spraying. Iron sulphate has affected the plants during the night when the relative humidity was high. C, plants one week after spraying. In the unsprayed pot, a, the mustard plants have completely outgrown the oat plants. The iron-sulphate spray has not been able to prevent some of the mustard plants from flowering, while the sulphuric-acid spray has absolutely killed the mustard without harming the oat plants, which are growing vigorously.

Plants grown in dry soil always showed perfectly unharmed leaves. The epidermis was green under the crystals and the leaves were turgid. Plants from medium moist soil appeared to be the most susceptible, as they frequently showed black spots under the crystals. However, the leaves rarely lost their turgor. After the crystals were formed, there seemed to be almost no increase in the injurious effect of the spray on the leaves. The crystals adhered rather loosely to the surface of the leaf. During the following night, when the relative humidity rose to 100 per cent, the action of the spray seemed to continue. By the next morning the effect was very marked, especially on plants grown in medium moist or wet soil. The surface of the leaves under the crystals was a deep black and the discoloration had reached the lower surface of the leaves. Where the crystals were close together, the whole leaf was black and dead. The plants of the dry soil showed greater resistance. Three to four days elapsed after spraying before they were completely destroyed.

In the dry chamber where the humidity was around 30 per cent, the effect of the spray was still less. Plants grown in dry soil were completely unharmed after 24 hours, in spite of numerous crystals covering the leaves. The plants of the moist and wet soil series were more susceptible to the spray and showed some slight injury.

INFLUENCE OF RELATIVE HUMIDITY ON THE EFFECT OF THE SULPHURIC-ACID SPRAY

The action of sulphuric acid was very similar under all conditions of humidity under which it was applied. In the moist atmosphere the action was somewhat delayed, but after two hours exposure to a temperature of 20° C. the leaves were very flaccid and yellow spots appeared on 30 to 50 per cent of the leaf area. Petioles and stems were also affected similarly. On plants sprayed in the greenhouse the effect was visible much sooner, as the water from the dilute spray evaporated, so that the concentration of the acid increased. After a period of five to six hours the leaves were almost completely dried out. In the dry atmosphere the effect of the spray was visible sooner after its application. The leaves of the mustard plants were dead and dry after four hours.

INFLUENCE OF TEMPERATURE ON THE EFFECT OF SPRAYS

In the second series of experiments an attempt was made to determine the influence of the temperature on the effect of the sprays. A 2 per cent solution of sulphuric acid and a 15 per cent solution of iron sulphate were used. As the experiments under higher temperature were carried out inside the greenhouse, and those under lower temperature were carried out in the open during cool days, the influence of the temperature was interfered with by the influence of the humidity of the air. However, under field conditions these factors are inseparable.

INFLUENCE OF TEMPERATURE ON THE EFFECT OF IRON SULPHATE SPRAY

At an average temperature of 6° C. and a relative humidity of 78 per cent the action of a solution of iron sulphate had a greater effect than at 30° and a relative humidity of 52 per cent. At the

lower temperature the spray did not evaporate in four hours, so that the crystals when formed had blackened the leaves. At the lower temperature the leaves frequently became flaccid and blackened, which rarely was the case at higher temperature, when the crystals were formed after 15 to 20 minutes.

INFLUENCE OF TEMPERATURE ON THE EFFECT OF SULPHURIC-ACID SPRAY

The action of sulphuric acid was very markedly affected by the temperature. In medium moist soil, the action at the higher temperature, 30° C., was very rapid indeed. After 15 minutes the leaves began to wilt and small yellow spots appeared. In 30 minutes the petioles turned yellow and the stems began to bend over. After one hour the leaves were completely wilted and 50 per cent of the leaf area was yellow or brown. Four hours was time enough to dry the leaves. The plants in the dry soil were affected somewhat more slowly than those in the wet or medium moist soil, but after an hour the difference between the series was hardly detectable. At the lower temperature, 6° C., the action was much delayed. Wilting was not observed until two hours after spraying. Small yellow spots appeared at the same time. After five hours the effect was not quite so marked as after one hour at 30° C. After 24 hours the plants were dead but not dry.

INFLUENCE OF RAIN ON THE EFFECT OF THE SPRAYS

Rain, falling after spraying is performed, may diminish or inhibit the effect of the operation, as the sprayed solutions will be washed off the plants. However, if the destruction of the plants by the sprays has proceeded beyond recovery when the rain occurs, the influence of the rain is negligible.

Experiments were conducted to test the time necessary for a spray to injure the plants beyond recovery. The plants were sprayed inside of the greenhouse and after a certain time, ranging from 30 minutes to 6 hours, sprinkled with a watering can. The temperature of the greenhouse was around 20° C. and the relative humidity around 55 per cent. It was found that plants sprayed with a 15 per cent solution of iron sulphate were almost unharmed when the spray was washed off three to six hours later. The leaves on plants grown in moist soil were blackened to some extent, but all plants recovered completely. Plants sprayed with a 2 per cent solution of sulphuric acid recovered when sprinkled 30 minutes after they had been sprayed, but if the sprinkling was done one hour after spraying the plants died. However, on the recovering plants, the top buds were destroyed, which means a great check to the plants.

ADDITIONAL TEST OF THE EFFECT OF SOLUTIONS OF IRON SULPHATE AND SULPHURIC ACID ON PLANT TISSUES

In order to obtain additional evidence of the relative rapidity of the action of iron sulphate and sulphuric acid upon plant tissues the following experiments were performed with leaves of water weed, *Elodea canadensis* Rich. The easily observed streaming of the protoplasm in the leaf cells of *Elodea* gives a good indication of unharmed cells. Injury to the cells results in a cessation of the protoplasmic movement.

Leaves of the plant were mounted on cover glasses by using vaseline at one end. Hollow-ground slides such as are used in bacteriological work were used as containers for the solution to be tested, so that the leaf could be observed at any time during the experiment. The time of cessation of streaming was noted, after which the leaf was transferred to a solution of half the strength for half an hour and thereafter into water. After half an hour the leaves were reexamined and the effect noted. The following solutions were used: 10 and 20 per cent solutions of cane sugar, 5 and 10 per cent solutions of iron sulphate, and 0.5, 1, and 1.5 per cent solutions of sulphuric acid. The experiment was conducted at room temperature, about 20° C.

In a 10 per cent solution of cane sugar the streaming continued undisturbed for six hours. No plasmolysis was observed during this time. In a 20 per cent solution plasmolysis was induced and streaming ceased after about 20 minutes, probably owing to the increased viscosity of the cytoplasm. After the cells were transferred to a weaker solution of sugar and then to water they were deplasmolysed and some streaming was noted, indicating that the cells were unharmed. Cells were kept plasmolysed up to four hours with similar results. Plasmolysis did not harm the cells under these conditions.

In a 5 per cent solution of iron sulphate the cells did not become plasmolysed. The streaming began to decrease after about one hour but continued for about two hours. After the cells were placed in water they were tested in a 20 per cent solution of cane sugar. Some cells became plasmolysed after being in the solution of iron sulphate for three hours. The chloroplasts were still green but appeared massed together. A 10 per cent solution of iron sulphate caused plasmolysis after 10 to 15 minutes, but streaming continued up to two hours. The cells were not deplasmolysed when placed in water after they had remained in a 10 per cent solution of iron sulphate for three hours. The chloroplasts were of a natural color and size but clustered together in the plasmolysed cells.

Streaming was never observed when the cells were placed in a 1.5 per cent solution of sulphuric acid. The time necessary to place the slide under the microscope was apparently long enough to stop the movement. After a minute or more the chloroplasts became yellow. If the leaves were then placed in water and thereafter into a 20 per cent solution of cane sugar no plasmolysis was observed. In a 1 per cent solution of sulphuric acid the streaming was observed for 20 to 30 seconds. After two minutes the chloroplasts became yellow and the cells were dead. When the leaves were placed in a 0.5 per cent solution of sulphuric acid, streaming was observed for from two to four minutes. After five to seven minutes the chloroplasts were yellow and the cells showed no sign of plasmolysis when placed in a 20 per cent solution of cane sugar. In no case did a sulphuric-acid solution cause plasmolysis of the cells.

ANALYSES OF PLANTS

In a previous study the writer (*3*) found a correlation between the chemical composition of plants and the strength of sulphuric acid necessary to kill the plants. It was also found that the farther away they were from the cotyledons the more compact was the structure of the leaves of pot-grown mustard plants. In order to

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determine whether the difference in the chemical composition of the later formed leaves was sufficient to explain the frequently reported hardiness of the plants in the late rosette stage, against sprays, leaves were analyzed for dry weight and ash content.

The mustard plants from five cultures of each series were harvested for analysis. As each culture had 8 plants, 40 plants of each series were analyzed. The green weight, dry weight, and total ash content were determined for the successive leaves of the plant beginning at the cotyledons. The results of these analyses are recorded in Table 5. This table shows that the plants in the dry soil had the largest amount of dry matter and ash, expressed in per cent of green weight.

TABLE 5.—*Analyses of Brassica arvensis plants in rosette stage, grown in greenhouse from February 8 to March 15, 1926*

[Figures represent total weight of 40 plants]

Leaves analyzed	Green weight (grams)	Dry weight (grams)	Per-centage dry weight	Ash (grams)	Ash in per-centage of green weight	Ash in per-centage of dry weight
Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity):						
Cotyledons	3.75	0.370	9.87	0.1155	3.08	31.21
First leaf	5.5	.568	10.32	.1575	2.86	27.72
Second leaf	8.7	1.122	12.89	.246	2.83	21.92
Third leaf	6.1	.994	16.30	.166	2.72	16.70
Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity):						
Cotyledons	8.4	.534	6.36	.185	2.20	34.64
First leaf	12.9	1.022	7.92	.2655	2.06	25.98
Second leaf	23.4	2.017	8.62	.475	2.02	23.55
Third leaf	22.9	2.238	9.77	.4095	1.78	18.28
Fourth leaf	10.0	1.181	11.81	.175	1.75	14.81
Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity):						
Cotyledons	8.9	.572	6.62	.191	2.14	33.39
First leaf	11.0	.956	8.96	.241	2.19	25.31
Second leaf	17.7	1.615	9.12	.3795	2.14	23.44
Third leaf	21.8	2.287	10.48	.425	1.95	18.58
Fourth leaf	12.0	1.522	12.68	.220	1.83	14.45

TABLE 6.—*Analyses of Brassica arvensis plants in rosette stage, grown in greenhouse from March 22 to April 22, 1926*

[Figures represent total weight of 40 plants]

Leaves analyzed	Green weight (grams)	Dry weight (grams)	Per centage of dry weight	Ash (grams)	Ash in per centage of green weight	Ash in per centage of dry weight
Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity):						
Cotyledons	3.5	0.335	9.57	0.105	3.00	31.34
First leaf	4.9	.537	10.96	.128	2.61	23.83
Second leaf	7.1	.852	12.00	.172	2.42	20.18
Third leaf	6.0	.806	13.43	.144	2.40	17.87
Fourth leaf	2.7	.382	14.15	.059	2.18	15.45
Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity):						
Cotyledons	9.0	.536	5.95	.172	1.91	32.08
First leaf	13.8	1.086	7.87	.245	1.78	22.56
Second leaf	21.9	1.755	8.01	.334	1.52	19.03
Third leaf	25.1	2.507	9.98	.425	1.69	19.95
Fourth leaf	16.4	1.945	11.86	.312	1.90	16.04
Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity):						
Cotyledons	7.8	.524	6.72	.175	2.24	33.39
First leaf	12.8	1.080	8.44	.247	1.93	22.83
Second leaf	19.9	1.780	8.94	.396	1.99	22.25
Third leaf	20.8	2.252	10.82	.391	1.87	17.37
Fourth leaf	13.5	1.630	12.07	.241	1.78	14.78

TABLE 7.—*Amount of dry matter and ash per unit of leaf area of Brassica arvensis plants*

[Figures are based on 40 plants]

Leaves analyzed	Area (sq. dcm.)	Dry matter (grams)	Ash (grams)	Quantity per sq. dcm. of leaf area of—	
				Dry matter (grams)	Ash (grams)
Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity).					
Cotyledons	0.99	0.335	0.105	0.338	0.016
First leaf	1.42	.537	.128	.378	.090
Second leaf	3.16	.852	.172	.269	.054
Third leaf	3.16	.806	.144	.255	.045
Fourth leaf	1.10	.382	.059	.347	.054
Total or average	9.83	2.912	.608	.296	.062
Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity):					
Cotyledons	1.98	.536	.172	.271	.087
First leaf	3.67	1.086	.245	.296	.067
Second leaf	7.95	1.755	.334	.221	.042
Third leaf	10.85	2.507	.425	.231	.039
Fourth leaf	8.28	1.945	.312	.234	.037
Total or average	32.73	7.829	1.488	.239	.045
Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity):					
Cotyledons	1.82	.524	.175	.288	.096
First leaf	4.00	1.080	.247	.270	.061
Second leaf	6.70	1.780	.396	.265	.059
Third leaf	7.96	2.252	.391	.283	.049
Fourth leaf	6.52	1.630	.241	.250	.037
Total or average	27.00	7.266	1.450	.269	.054

The plants in the medium moist soil showed the lowest content of dry matter and those in the wet soil an intermediate amount. However, the difference between plants of medium moist and wet soil was not very marked. Expressed in per centage of green weight, the dry matter of the leaves increased from the cotyledons upward while the ash content decreased.

Analyses of plants grown in the second experiment checked very closely with those of the first experiment. (See Table 6.)

The analyses of the plants in the first experiment gave results which show a marked difference in the chemical composition of the leaves, depending on their position above the cotyledons. But figures expressing percentages may give a false impression. If the action of sulphuric acid is diminished or inhibited by its absorption into the plant tissue (this action depends upon the amount of dry matter and ash of the leaves), it is obvious that it is the amount of dry matter and ash per unit of area which is important. The spray is always applied in a given quantity per unit area. Thus it was necessary to measure the leaf area in order to calculate the amount of dry matter and ash per unit of area. In the second experiment the leaf areas were determined by measuring tracings of the leaves with a planimeter. The results are recorded in Table 7.

Table 7 shows that there was a difference between the plants of the three series. Plants grown in the dry soil had the highest average amount of dry matter and ash per square decimeter of leaf area. Plants grown in medium moist soil had the lowest and those in

wet soil had an intermediate amount of dry matter and ash.   Thus far the figures agree with those expressing the amount in percentage of green weight.   However, instead of increasing from the cotyledons upward, the dry matter per square decimeter actually decreases. The ash content also decreases, even to a more marked degree.   The results of the analyses recorded in Table 7 seem to indicate that the resistance of the mustard plants, in late rosette stage, can not be due to the increased amount of dry matter and ash in the upper leaves of the plants.

DISCUSSION

THE EFFECT OF THE SPRAYS

The experiments show that there is a very marked difference in the action of the sprays used.   The cells of a plant are able to endure a relatively high concentration of iron sulphate for a considerable time, while a very dilute solution of sulphuric acid kills the cells almost instantly.   On the other hand, iron sulphate seems to be able to act for some distance.   For instance, if plants are sprayed, the spray is seen to adhere to the leaves, but rarely to the petioles or the stems.   Nevertheless, after some time, especially on plants placed in the moist chamber, it was found that both petioles and stems were black and killed.   The salt seems to move through the tissues of the leaves.   With sulphuric acid this was never observed, at least not with the quantities used as sprays, the acid acting solely at the place where it hits the leaf.   On the other hand, it seems to adhere more readily to petioles and stems, as these were observed to be killed as rapidly as the leaves.   It is shown, however, that sulphuric acid under certain circumstances may move through the leaves.   If the tip of a leaf is placed in a solution of the acid, the whole plant will be killed.   In the quantities applied as sprays the acid seems to be absorbed by the tissues without being transported to other parts of the plants.

The action of iron sulphate upon plant tissues is not clearly under-stood.   Several theories have been advanced.   Olive (27) believes that "death is due to osmotic properties rather than to absorption of the chemical into the leaves."   The water, according to his theory, is drawn out of the leaves by the flakes of dried salt on the surface.   He believes drying of the solution to be a necessary process. On the contrary, Schultz (34), citing some investigations by Stender (35), states that leaves are unharmed after being plasmolysed for several hours by various salt solutions, if the salt is then washed off. Earlier experiments by the writer (3) in which plant tissues were treated with strong solutions of sodium chloride, confirm these results. Schultz finds that iron sulphate has a certain harmful effect, but declares that he does not know how it acts.

The blackening of the leaves of sprayed plants is explained by Olive (27) as being due to the formation of sulphides in the leaf or "union of   *   *   *   sulphate with the living substance."   Plants of the mustard family are characterized by the presence of mustard oils which contain a relatively high per cent of sulphur.   This fact is probably the basis for Olive's interpretation of the action of iron sulphate.   Schultz (34), however, denies that this reaction takes place. He believes that the blackening of the leaves is due to a reaction between iron and tannic acid of the tissues.   Whatever the action

may be, one thing is clear, and that is that the action is a slow one. Leaves of *Elodea* endured a 10 per cent solution for two hours.

The experiments show that a solution of iron sulphate may easily kill the tissues without causing plasmolysis. A 5 per cent solution completely destroyed the mustard plants in 24 hours in the moist chamber. But as such a solution did not cause plasmolysis in leaves of *Elodea*, even though most of the cells were killed within three hours, probably it acted in a similar way on leaves of mustard. In the moist chamber no evaporation took place, so that an increase in concentration was out of the question. The experiments thus support the view of Schultz that it is the chemical action of the spray that kills the plants.

The action of sulphuric acid is easier to interpret. It is the high hydrogen-ion concentration which is injurious. Brenner (*10, 11*) has studied extensively the effect of various acids on plant cells. He found them injurious in proportion to their acidity, i. e., in proportion to the actual hydrogen-ion concentration of the solution. He placed pieces of epidermis from several plants in acids of various strengths and tested their effect after a certain time. For instance, he found that a $\frac{M}{50}$ solution of sulphuric acid, equivalent roughly to a solution of 0.2 per cent killed cells of *Brassica oleracea* in two minutes. A $\frac{M}{1300}$ solution killed the cells in four hours. The hydrogen-ion concentration of this solution he gives as 1.4×10^{-3}, Expressed in a more common term of hydrogen-ion concentration, the acidity was approximately pH 2.85. Calculated in percentage this solution contained about 0.0075 per cent sulphuric acid. These figures, as well as the experiments with *Elodea* reported in this paper, show clearly that the protoplasm is very susceptible to an acid solution. However, it is not possible to destroy weed plants with a sulphuric-acid solution of 0.2 per cent strength, in spite of the fact that Brenner has shown that it will kill cells in two minutes. In these experiments a 2 per cent solution was necessary; in field trials in spring-sown grain crops a 3.5 to 4 per cent solution was required. Rabaté (*31*) found that a 10 per cent solution was just strong enough to kill weeds in winter wheat sprayed in early spring. This makes it clear that plants are able to absorb some acid and neutralize it, so that they will be killed only when the absorbing capacity has been exceeded. Only a small amount can adhere to the plants. The necessity of increased strength of spray when winter-grown weeds are to be eradicated indicates that the difference in resistance is to be looked for in the difference in anatomical structure of plants grown under various conditions. The illustrations in Figure 5 are cross sections of leaves of *Brassica arvensis* grown under various conditions. Plants grown under the most severe conditions have the most compact leaf structure. It is the cell walls which are affected. This makes it clear that the protecting capacity is situated in the cell walls, which seem to be able to absorb and neutralize at least some of the acid.

The great resistance of plants to sprays frequently observed in the late rosette stage, seems not, as far as these experiments show, to be due to the increased amount of dry matter and ash in the upper leaves, as earlier experiments suggested. There is actually a decrease

in dry matter and ash content per unit of leaf area from the lower leaves upward. However, this increased resistance is reported, especially when sprays of salt solutions of various kinds have been used. Resistance to such sprays is a question to a large extent of the thickness and modification of the outer cell walls of the epidermis, which are greatly influenced by periods of drouth such as very commonly occur in spring and early summer. Still another explanation may be found. As long as the plants are small, the leaves are growing more or less horizontally. A spray at this time will adhere to the plants rather easily and in large amounts. When the plants grow larger and are in competition with grain plants the upper leaves grow more nearly vertically. A spray applied under these conditions adheres with more difficulty than on horizontal leaves and the amount of spray adhering per unit of leaf area will be less. The larger the leaves
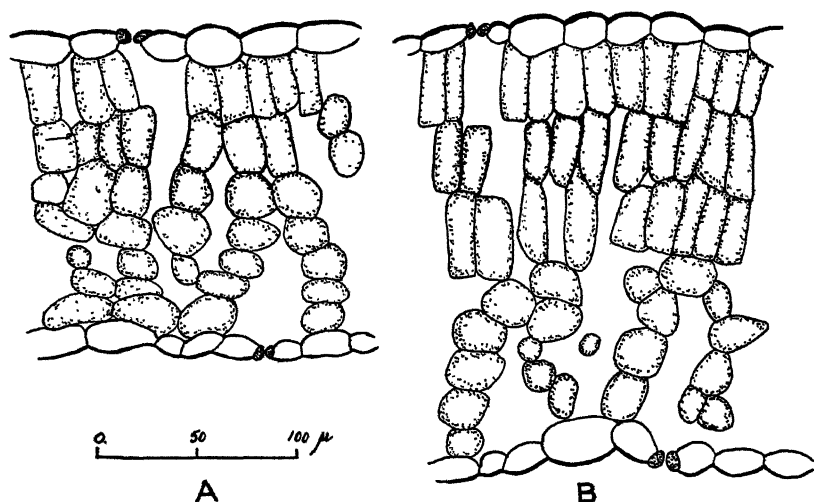
Fig. 5.—Cross section of leaves of *Brassica arvensis:* A, leaf of plant grown in medium moist soil, showing rather loose structure, weakly developed palisade tissue, and large air spaces; B, leaf of plant grown in dry soil, the structure of which is more compact, which accounts for the greater resistance of the plants to sprays

Both drawings were made with the aid of a camera lucida and are of the same magnification

of crowded plants, the more will these plants protect each other. Thus early spraying is always more destructive to the weeds.

INFLUENCE OF WEATHER ON THE EFFECT OF SPRAYS

The experiments confirm the reports of field trials of weed eradication, namely, that rapidly growing plants are most susceptible to sprays. The most suitable weather conditions for spraying, according to general opinion, obtain on a dry, sunshiny, calm day. It is significant that Bolley (6) is the only one who recommends humid weather rather than dry weather for the application of weed sprays. Working in North Dakota he found the wettest and most rapidly growing condition to be the most satisfactory, and states that "it is useless to expect desirable results by spraying in droughty, windy weather." He used several salt solutions as sprays.

Experiments described in the present work fully confirm the statement of Bolley. The action of a solution of iron sulphate under dry

conditions is very weak, especially on plants grown in dry soil. The salt crystallizes before the leaves are injured by the spray. In a moist atmosphere the spray acts rather efficiently. It is clear then that iron sulphate when sprayed under dry conditions begins to act when the relative humidity has increased to a certain point, which probably is near 100 per cent or at the dew point. For that reason good results may generally be expected in humid regions, and in dry regions only if the relative humidity happens to be high. With this point clear, it is of interest to examine the relative humidity in the agricultural regions of the United States during May and June, the time when weed sprays are usually applied. Day (*12*) in an extensive report on the relative humidity of the United States, shows very clearly that with the exception of the coastal regions, the relative humidity is very low during the spring and early summer.

Ward (*38*) in a paper discussing the relative humidity of the United States cites Hann (*15*), who states that the relative humidity of the New England States, one of the humid regions of the United States, is lower than it is in western Europe. He tries to explain this condition as an effect of prevailing winds. This low relative humidity explains why the use of iron sulphate and other salt sprays against weeds have given much better results in Europe than in the United States.

The action of sulphuric-acid spray upon plant tissues is favored by warm and dry weather. The warmer and dryer the weather the better are the results obtained.

The protoplasm is killed instantly when it comes in contact with sulphuric acid of the strengths used in sprays. The time elapsing from the moment the spray adheres to the plant until the tissues are killed depends upon the rate at which the acid penetrates the tissues. The rate of diffusion increases rather rapidly with rise in temperature. The fact that the rate of action of the spray increased about five times when the temperature was raised from 6° to 30° C. may be accounted for chiefly by the increased rate of diffusion. The action of the acid on the tissues is a chemical one. Rise in temperature increases the velocity of a chemical reaction still more than that of a physical one. Thus an increase in temperature accelerates the chemical action of the spray still more than its diffusion into the tissues. At the higher temperature employed in these experiments evaporation is very much higher than at the lower one. Evaporation of water from the spray increases the concentration of the sulphuric acid and thus increases its action. Another effect of the increased evaporation at the higher temperature is that the sprayed parts of the plant begin to dry up as soon as the protoplasm is killed, or as soon as the plant becomes flaccid. This drying rapidly increases the visible effect of the spray. Taking into consideration these effects of an increase in temperature on the action of the sulphuric-acid spray, its rapidly increasing effectiveness with rise in temperature is readily accounted for.

SUMMARY

Plants of field mustard, *Brassica arvensis* (L.) Ktze, and Cornellian oats, *Avena sativa* L. were grown in pot cultures in the greenhouse.

The soil moisture of the cultures was kept at 15, 30, and 45 per cent of the moisture-holding capacity of the soil. A watering

system giving an even distribution of moisture in the soil was adopted. Mustard plants grown in the dry soil were found to have a much more compact anatomical structure than those grown in medium moist or wet soil.

The plants were sprayed with solutions of iron sulphate varying in strength from 5 to 15 per cent and with solutions of suphuric acid, varying in strength from 1 to 2 per cent. The sprayed amount was 1 gm. per square decimeter (1,000 liters per hectare, or 107 gallons per acre).

The sprayed plants were exposed to three conditions of atmospheric humidity, namely, about 30, 60, and 100 per cent of relative humidity. A solution of iron sulphate was found to be most destructive in an atmosphere containing about 100 per cent relative humidity. Under such conditions a 5 per cent solution completely killed the mustard plants in 24 hours. In dry air, with a relative humidity from 30 to 60 per cent, the solution of iron sulphate sprayed upon the plants evaporated rapidly and salt crystals were formed on the surface of the leaves without injury to the plants. When the relative humidity was allowed to increase to about 100 per cent, the plants were soon killed. This action was easily followed on plants grown in dry soil, while plants grown in moist soil were sometimes injured before the crystals were formed. Solutions up to 15 per cent strength were used without any different effect. As the relative humidity in the United States generally is very low during May and June a spray of iron sulphate will have but a slight effect on hardy plants.

When a solution of sulphuric acid was sprayed on the plants, the mustard plants were killed under all conditions of humidity, but best results were obtained in dry air. Plants grown in moist soil were killed off by a 1.5 per cent solution, while plants grown in dry soil required a 2 per cent solution to destroy them completely. This results indicates that the latter plants were able to absorb some acid without being injured permanently. As a spray of sulphuric acid gives the best results in dry air it is a spray to be recommended for dry regions.

Temperature had a marked influence upon the effect of the sprays. At 30° C. a 2 per cent solution of sulphuric acid killed the plants in one hour, while at 6° the same effect was obtained only after five hours. A 15 per cent solution of iron sulphate was more effective when sprayed at a lower temperature, as the evaporation of the water and crystallization of the sulphate was very slow giving the solution a longer time to act. In no case did a solution of iron sulphate kill the plants in less than 24 hours.

Artificial rain, produced by sprinkling, applied to the plants one hour after they had been sprayed with a 2 per cent solution of sulphuric acid failed to decrease the effect of the spray. Plants grown in moist soil sprayed with a 15 per cent solution of iron sulphate were but slightly harmed when "rain" was applied six hours after spraying. Plants grown in dry soil were unharmed.

In an additional test of the relative rapidity with which solutions of iron sulphate and sulphuric acid act upon living plant cells it was found that protoplasmic streaming in the leaves of *Elodea canadensis* Rich. continued for two hours in a 10 per cent solution of iron sulphate but ceased in 30 seconds in a 1 per cent solution of sulphuric

acid. The cells were killed after having been kept in the iron sulphate solution for three hours or in the sulphuric acid for two minutes.

Analyses of plants indicate that, the great resistance to sprays exhibited by plants in the late rosette stage, can not be explained by the increased amount of ash and dry matter in the upper leaves. Other explanations are suggested.

The oat plants were not harmed by the sprays.

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THE WATER REQUIREMENT OF PLANTS AT AKRON, COLO.[1]

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INTRODUCTION

The term "water requirement" indicates the ratio of weight of water absorbed by the plant during its growth period to the weight of dry matter harvested. Only in the case of root crops is the weight of the underground parts included. Previous measurements at Akron, Colo., have been reported by Briggs and Shantz.[4] Their results include two crops grown in 1910, 31 in 1911, 44 in 1912, and 55 in 1913.[5]

The experiments were enlarged in 1914, 1915, and 1916 to include 68 sets of plants, but in 1917 this number was reduced to 29 sets. A few additional sets were grown each year as late-season crops or in specially constructed pots. The published data covered 132 sets of 6 pots each, while the data here presented comprise an additional 147 sets of 6 pots each. The total experiment represents 288 sets of more than 1,800 pots, covering the period 1911 to 1917. There are here included only those experiments which give relative values and are directly comparable, and these are combined in a final summary (Tables 33, 34, and 35). A weighted summary, which represents the mean value for a period of years for each crop and the relative water requirement for the different crops, as closely as they can be expressed from the measurements at hand, is also given. Estimates are made of the probable highest and lowest values which would have been recorded had each crop been grown continuously from 1908 to 1925. These estimates are based on evaporation and transpiration data. The experiments at Akron, Colo., were discontinued at the end of 1917.

METHODS

The methods used in conducting these experiments have been fully described in the publications already cited. Plants were grown in a screened inclosure to protect them from hail and birds (fig. 1), and the results thus obtained were compared with those obtained with plants freely exposed, and also with plants grown in fields of grain.

The pots used contained about 115 kilograms of soil. Each pot was provided with a tight-fitting cover and sealed. A capillary tube was inserted through the corks to prevent the carrying in of rain water by suction resulting from rapid cooling during showers. The pots were weighed daily, or as often as necessary to insure their maintenance at the proper water content. The initial weight was taken as soon as the pots were planted and waxed. The pots were weighed with a spring balance. This balance was checked against a platform balance several times during each weighing, for temperature
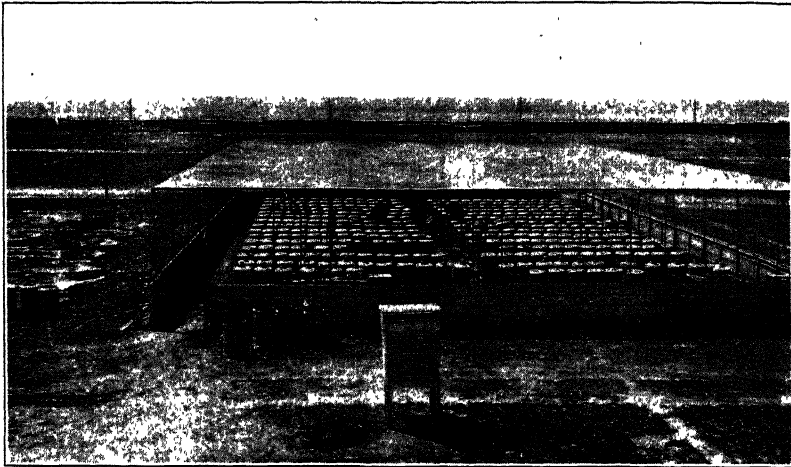
FIG. 1.—A general view at planting time of the shelter in which water requirement measurements at Akron were made. Photographed May 8, 1914

and other changes, so that the results obtained were accurate to within one-fifth of a kilogram. Water was added from calibrated flasks with the neck cut to deliver 2 liters.

Fertilizer composed of 25 parts per million of PO_4, 50 parts per million of NO_3, and 33 parts per million of K was added to each pot four times during the growth period. In 1911 and 1914 twice this amount was added. One-fourth of the fertilizer was added in 2 liters of water and was followed immediately by an additional 2 liters of water. Check determinations showed no difference in the water requirement of fertilized and unfertilized pots.

Akron, Colo., is located on the high plains, and the national vegetation consists of a relatively pure short grass cover. (See fig. 2.)

Complete records of weather factors were taken, including solar radiation, depression of wet bulb thermometer, air temperature, wind velocity, and evaporation from a free water surface.[6][7]

[6] BRIGGS, L. J., and SHANTZ, H. L. HOURLY TRANSPIRATION RATE ON CLEAR DAYS AS DETERMINED BY CYCLIC ENVIRONMENTAL FACTORS. Jour. Agr. Research 5: 583–650. illus. 1915.
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EFFECT OF THE SHELTER

The results presented in this paper were obtained in a screened inclosure covered with No. 21 galvanized wire netting of ⅜-inch mesh. The previous measurements [8] show that the light was reduced about 20 per cent by the inclosure. The water-requirement measurements have led to the conclusion that pots sunk in trenches, surrounded by a field of grain, have a water requirement of about 10 per cent above wheat grown in an inclosure, and 10 per cent below that of wheat grown outside the inclosure in a freely exposed position.
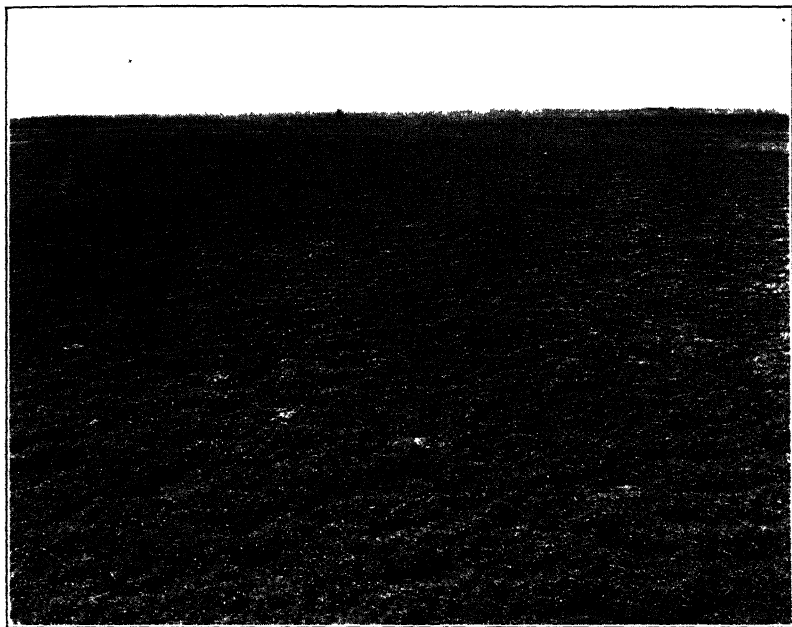
FIG. 2.—General view of the natural vegetation at Akron, Colo. A relatively pure cover of grama grass (*Bouteloua gracilis*) and buffalo grass (*Buchloe dactyloides*). Photographed April 18, 1910

As the result of a long series of observations, measurements for 1911, 1913, 1914, 1915, 1916, and 1917 are now available (Table 1). These measurements show a wide variation, and many factors must be taken into account in their interpretation. The conditions in the field were probably more favorable at times than those in the shelter, especially for such warm weather crops as sorghums and millets. Observations in the shelter were made under most favorable conditions, since the plants were protected from excessively high winds and from damage by hail, wind, or birds (fig. 3). In the field, on the other hand, plants were subject to all the variable and inclement conditions of the weather, so that the measurements show a much wider variation and a much greater probable error than those grown in the shelter.

While the plants grown in the open were also exposed they were watched much more closely and were protected to some extent against excessive storms. The values here presented are more extensive than those cited by Briggs and Shantz in the 1913 measurements. The measurements presented in Table 1 show that the water

[8] BRIGGS, L. J., and SHANTZ, H. L. Op. cit. 1914. p. 3.

requirement in the field was 10 per cent higher than that in the screened inclosure, which is in exact accord with the results previously obtained. For the freely exposed plants, however, the water requirement was only 3 per cent above that in the field or 13 per cent above the crops in the shelter. It seems safe to assume that had these experiments been carried on in the open, unprotected, and with the same exposure as field plots, the water requirement would have been about 10 per cent higher than at present recorded. Table 1 gives the results obtained under the three locations.
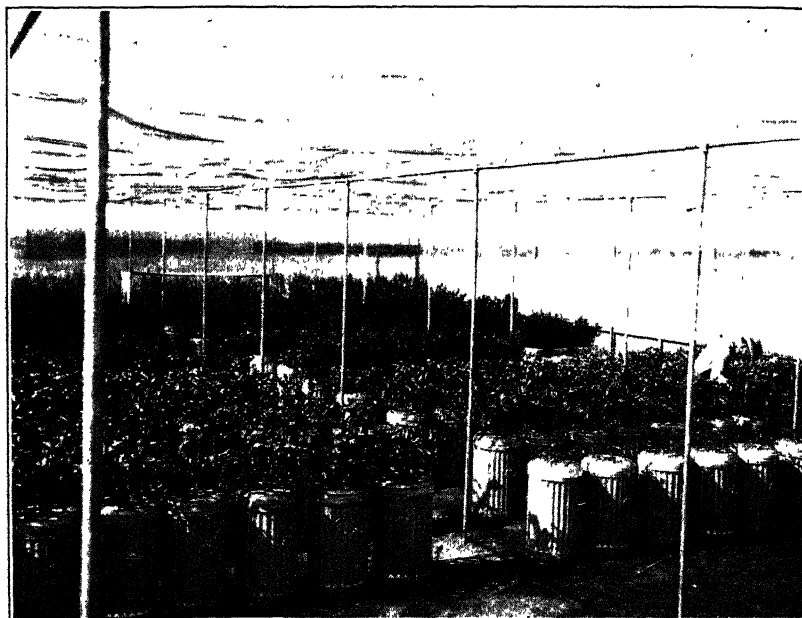
FIG. 3.—A general view in midseason of the shelter in which the water requirement measurements at Akron were made. Photographed July 13, 1912

TABLE 1.—*Effect of exposure on water requirement, as shown by experiments with plants grown in the open but slightly protected, in the field, and under shelter, at Akron, Colo., field station*

Year	Crop	Water requirement of plants—					
		In open		In field		In shelter	
		Actual	Per cent	Actual	Per cent	Actual	Per cent
1911	Tumbleweed	275±7	99			277±4	100
1913	Wheat, Kubanka, C. I. 1440	627±5	126	562±6	113	496±5	100
	Alfalfa, Grimm, E-23	1030±12	124			834±8	100
1914	Wheat, Kubanka, C. I. 1440			625±20	121	518±6	100
	Sudan grass	455±9	115	402±6	102	394±4	100
	Alfalfa, Grimm, E-23	1039±19	117			890±6	100
	Millet, Kursk			287±6	97	295±2	100
1915	Wheat, Kubanka, C. I. 1440			361±3	89	405±3	100
	Sudan grass	287±8	110	290±5	112	260±3	100
	Alfalfa, Grimm, E-23	795±20	114			695±9	100
	Millet, Kursk			218±3	108	202±1	100
1916	Wheat, Kubanka C. I. 1440			908±50	143	636±14	100
	Sudan grass			377±10	88	426±3	100
	Alfalfa, Grimm, E-23	1095±25	105			1047±9	100
	Millet, Kursk			460±16	125	367±4	100
1917	Sudan grass	409±9	108			378±3	100
	Average		113		110		100

WATER-REQUIREMENT EXPERIMENTS, 1910 TO 1917

As already mentioned, the results of experiments from 1910 to 1913 have been published, only the final results being included in the summary in Tables 33, 34, and 35 of this discussion.

Many of the experiments from 1914 to 1916, which included 68 sets of plants used each year, covered special measurements not included in this paper. Only those which are comparable and which have a bearing on the relative water requirement of plants are here presented. To obtain the data presented in each table six determinations usually were made for each crop. These have been combined into an average for which a probable error has been calculated.

WATER-REQUIREMENT EXPERIMENTS, 1914

Sixty-one sets of plants were grown in 1914, which was an average year, the water requirement being approximately the same as the mean for the series of plants grown each year from 1911 to 1917. (Tables 27 and 28.) The evaporation also was practically the same as for the mean of the period 1911–1917 (Tables 31 and 32) and for the longer period 1908–1924 (Table 32). During this year special attention was given to alfalfas which were being tried out in the dry land regions, and to corn and wheat varieties. The results of the water-requirement measurements of the corn hybrids grown have already been presented.[9]

WATER REQUIREMENT OF WHEAT VARIETIES, 1914

Fourteen varieties of wheat were grown in the experiments in 1914 (Table 2) 5 of which were durums, and 8 were common varieties and 1 was a hybrid. The results, based on dry matter, are as follows:

Durum wheats:
    Beloturka_____ 458 ± 10
    Jumillo_____ 496 ± 10
    C. I. 4131_____ 507 ± 8
    Kubanka, C. I. 1440____ 518 ± 6
    C. I. 4082_____ 538 ± 5

    Average_____ 503 ± 8

Common wheats:
    Marquis, C. I. 3641_____ 498 ± 5
    Preston, C. I. 3328_____ 510 ± 3
    C. I. 4090_____ 567 ± 6

Common wheats—Continued.
    Galgalos, C. I. 2398_____ 624 ± 5
    C. I. 4087_____ 638 ± 2
    Pacific Bluestem_____ 679 ± 3
    C. I. 4103_____ 689 ± 8
    C. I. 4127_____ᵃ916 ± 15

    Average_____ 601 ± 5

Hybrids:
    Jumillo × Preston_____ 574 ± 2

    Average for series_____ 560 ± 6

ᵃ Omitted from average.

The water requirement of the durum wheats in 1914 was 16 per cent lower than the water-requirement measurements for the common wheats. The Beloturka variety gave the lowest water-requirement value, 458 ± 10. The next most efficient of the durum wheats was Jumillo. The standard variety, Kubanka, grown throughout the series of experiments, ranked above all the other varieties of durums, with the exception of an introduction from Peru, C. I. 4082, which had a value approximately 4 per cent higher than Kubanka. The value of Kubanka was 518 ± 6. The variation of the different varieties of durum wheats based on the lowest water-requirement value amounted to only 17 per cent.

⁹ BRIGGS, L. J., and SHANTZ, H. L. INFLUENCE OF HYBRIDIZATION AND CROSS-POLLINATION ON THE WATER REQUIREMENT OF PLANTS. Jour. Agr. Research 4: 391–402, illus. 1915.

TABLE 2.—*Water requirement based on grain and dry matter, of varieties of wheat at Akron, Colo., 1914*

Variety of wheat	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Kubanka, C.I.1440(*T.durum*).	May 9 to Aug. 6___	1	340. 6	126. 3	171. 0	37	1354	502
		2	245. 2	95. 3	123. 2	39	1293	502
		3	287. 3	108. 1	155. 5	38	1438	541
		4	329. 0	123. 9	173. 6	38	1401	528
		5	318. 8	119. 2	161. 7	37	1357	507
		6	313. 1	121. 7	165. 8	39	1362	530
Mean_____							1367±13	518±6
Galgalos, C. I. 2398 (*T. aestivum*)	May 9 to Aug. 11 _	7	292 5	91. 9	189. 3	31	2060	647
		8	305. 2	98. 9	192. 5	32	1946	631
		9	283. 9	96. 1	172. 9	34	1799	609
		10	278. 8	94. 3	166. 3	34	1764	596
		11	311. 8	101. 3	195. 5	32	1930	627
		12	318. 1	105. 8	201. 1	33	1901	632
Mean_____							1900±30	624±5
Pacific Bluestem C. I. 4067 (*T. aestivum*).	May 9 to Aug. 12__	13	352. 3	76 4	236. 4	22	3094	671
		14	315. 6	56. 0	220. 5	18	3938	699
		15	328. 4	66. 9	225. 4	20	3369	686
		16	335. 4	61. 4	223. 3	18	3637	666
		17	312. 4	61. 1	210. 7	20	3448	674
		18	326. 6	56. 9	221. 7	17	3896	679
Mean_____							3564±98	679±3
C. I. 4087 (*T. aestivum*)_____	May 9 to Aug. 4___	19	312. 4	88. 7	196. 2	28	2212	628
		20	272. 6	75. 8	175. 6	28	2317	644
		21	294. 5	95. 0	189. 2	32	1992	642
		22	296. 2	95. 9	189. 5	32	1976	640
		23	291. 1	94. 1	185. 1	32	1967	636
		24	267. 9	81. 8	171. 3	31	2094	639
Mean_____							2093±43	638±2
C. I. 4090, S. P. I. 36502 (*T. aestivum*).	May 9 to Aug. 4__	25	272. 5	101. 7	157. 3	37	1547	577
		26	260. 1	91. 6	146. 9	35	1604	565
		27	258. 0	90. 9	150. 0	35	1650	581
		28	271. 3	91. 7	154. 0	34	1679	568
		29	279. 5	94. 0	166. 1	34	1767	594
		30	324. 4	109. 7	168. 5	34	1536	519
Mean_____							1631±26	567±6
C. I. 4103 (*T. aestivum*)_____	May 9 to Aug 4__	31	220. 7	_____	157. 7	_____	_____	715
		32	218. 4	_____	146. 7	_____	_____	672
		33	239. 5	_____	158. 9	_____	_____	663
		34	242. 0	_____	167. 1	_____	_____	690
		35	247. 3	_____	165. 0	_____	_____	667
		36	214. 9	_____	155. 7	_____	_____	725
Mean_____								689±8
C. I. 4127 (*T. aestivum*)_____	May 9 to Aug. 4__	37	193. 8	_____	168. 0	_____	_____	867
		38	174. 9	_____	157. 4	_____	_____	900
		39	173. 3	_____	149. 7	_____	_____	864
		40	157. 8	_____	152. 4	_____	_____	966
		41	139. 1	_____	134. 4	_____	_____	966
		42	156. 8	_____	145. 8	_____	_____	930
Mean_____								916±15
Beloturka, C. I. 3705, S. P I. 35480 (*T. durum*).	May 9 to Aug. 11__	43	279. 0	102. 0	127. 3	37	1248	456
		44	301. 1	109. 9	152. 1	36	1384	505
		45	269. 5	96. 5	131. 9	36	1367	489
		46	232. 8	85. 2	94. 2	37	1106	405
		47	269. 3	103. 0	116. 1	38	1127	431
		48	306. 6	116. 7	141. 0	38	1208	460
Mean_____							1240±35	458±10
C. I. 4131 (from Siberia) (*T. durum*).	May 9 to Aug. 6__	49	158. 4	61. 2	71. 6	39	1170	452
		50	224. 9	92. 1	125. 2	41	1359	557
		51	221. 5	86. 4	114. 6	39	1326	517
		52	246. 6	98. 9	125. 8	40	1272	510
		53	236. 6	95. 2	117. 8	40	1237	498
		54	253. 8	95. 1	128. 7	37	1353	507
Mean_____							1286±23	507±8
C. I. 4082 (from Peru) (*T. durum*).	May 9 to Aug. 11__	55	252. 0	105. 0	144. 9	42	1380	575
		56	326. 4	123. 2	173. 9	38	1412	533
		57	313. 2	112. 4	168. 8	36	1502	539
		58	315. 2	125. 2	164. 2	40	1312	521
		59	295. 4	89. 8	154. 6	30	1722	523
		60	299. 4	112. 3	161 1	38	1435	538
Mean_____							1461±38	538±5

Table 2.—*Water requirement based on grain and dry matter, of varieties of wheat at Akron, Colo., 1914*—Continued

Variety of wheat	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P.ct*		
Marquis, C. I. 3641 (*T. aesti-vum*).	May 9 to Aug. 3__	61	242.6	84.6	120.6	35	1426	497
		62	226.8	76.3	111.3	34	1459	491
		63	195.0	64.6	102.3	33	1584	525
		64	225.4	72.3	105.3	32	1456	467
		65	194.4	68.1	98.4	35	1445	506
		66	187.9	65.1	94.1	35	1445	501
Mean____							1469±14	498±5
Jumillo, C. I. 1736 (*T. durum*)_	May 9 to Aug. 11_	67	209.7	83.0	110.4	40	1330	526
		68	240.7	91.7	129.0	38	1407	536
		69	252.5	92.7	120.5	37	1300	477
		70	265 7	98.6	134.8	37	1367	507
		71	262.3	95.5	125.0	36	1309	477
		72	334.8	132.1	151.8	39	1149	453
Mean____							1310±22	496±10
Jumillo×Preston (*T. durum*× *T. aestivum*).	May 9 to Aug. 1__	73	283.6	75.0	162.3	26	2164	572
		74	302.2	91.2	172.6	30	1893	571
		75	253.6	72.1	147.6	28	2047	582
		76	225.8	65.6	127.9	29	1950	566
		77	226.6	67.5	129.6	30	1920	572
		78	235.9	70.2	136.6	30	1946	579
Mean____							1987±30	574±2
Preston, C I. 3328 (*T. aesti-vum*).	May 9 to Aug. 3__	79	237.4	78.4	118.0	33	1505	497
		80	261.3	81.1	132.9	31	1639	509
		81	281.4	94.7	147.8	34	1561	525
		82	215.8	75.5	110.8	35	1468	513
		83	249.2	87.0	129.6	35	1490	520
		84	224.3	79.4	111.7	35	1407	498
Mean____							1512±22	510±3

Of the common wheats, the lowest water-requirement value, 498 ± 5, was obtained from Marquis; this was 8 per cent higher than the value obtained from the most efficient durum. The other varieties ranged in value up to 38 per cent above Marquis. In this discussion C. I. 4127, which is a fall variety and produced only rosettes in the experiments, has been eliminated from consideration and its value has been omitted in computing the average. One hybrid between a durum and a common wheat was included. This hybrid showed a water requirement 14 per cent above the mean water requirement of the two parents, 16 per cent above the durum parent and 13 per cent above the common parent.[10]

The water requirement of wheats, based on grain production, is as follows:

Durum:
Beloturka_____ 1240±35
C. I. 4131_____ 1286±23
Jumillo_____ 1310±22
Kubanka, C. I. 1440___ 1367±13
C. I. 4082_____ 1461±38

Average_____ 1333±28

Common:
Marquis, C. I. 3641____ 1469±14
Preston, C. I. 3328_____ 1512±22

Common—Continued.
C. I. 4090_____ 1631±26
Galgalos, C. I. 2398____ 1900±30
C. I. 4087_____ 2093±43
Pacific Bluestem_____ 3564±98

Average_____ 2028±48

Hybrids:
Jumillo×Preston_____ 1987±30

Average for series____ 1735±39

[10] Briggs, L. J., and Shantz, H. L.   Op. cit.   1915.

Arranged in order of increasing water requirement the order of common wheat varieties is about the same, whether the results are based on total dry matter or grain only. The durum wheats show decided superiority over the common wheats, the lowest of the latter series exceeding in water requirement the highest durum. The durums have a water requirement only 66 per cent of the average of the common wheats. In other words, the grain yield is proportionately higher in the durum wheats than in the common wheats.

The water requirement of the hybrid based on grain was far above either parent, and about 41 per cent above the mean of the two parents.

WATER REQUIREMENT OF OATS, BARLEY, AND RYE, 1914

Two varieties of oats were grown in 1914, Swedish Select having a water requirement of 599 ± 2, Burt 615 ± 6. These two varieties showed less than 3 per cent difference in water requirement. Oats has a water requirement 8 per cent higher than wheat. On the basis of grain produced, Swedish Select had a water requirement of 1421 ± 8 and Burt of 1483 ± 31, or about the same as the better common wheats.

Barley gave a water requirement of 501 ± 5 and rye of 622 ± 7. Rye, therefore, shows a higher water requirement than any of the groups of small grains, and is followed in turn by oats, the common varieties of wheat, and the durums. On the basis of grain production, barley had a water requirement of 1179 ± 28 and rye of 2291 ± 54. With the exception of Pacific Bluestem, one of the common wheats, barley is the most efficient and rye the least efficient of any of the small grains in the use of water. The water-requirement measurements of oats, barley, and rye as obtained in the Akron measurements are shown in Table 3.

TABLE 3.—*Water requirement, based on grain and dry matter, of oats, barley, and rye at Akron, Colo., 1914*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Barley, Hannchen, C. I. 531	May 9 to July 25__	85	186.8	81.4	89.6	44	1107	480
(*Hordeum distichon*).		86	187.5	80.8	91.6	43	1134	489
		87	187.0	79.1	95.9	42	1212	513
		88	184.4	78.6	91.4	43	1163	496
		89	177.3	80.7	88.1	46	1092	497
		90	168.3	65.4	89.4	39	1367	531
Mean___							1179 ± 28	501 ± 5
Oats, Swedish Select, C. I.	May 9 to Aug. 1__	91	234.0	97.1	138.8	41	1429	593
134 (*Avena sativa*).		92	254.2	108.2	152.9	43	1413	601
		93	269.2	110.8	161.7	41	1459	601
		94	255.9	107.4	154.8	42	1441	605
		95	288.2	119.7	170.0	42	1420	590
		96	285.3	126.3	172.5	44	1366	605
Mean___							1421 ± 8	599 ± 2
Oats, Burt, C. I. 293 (*A. sativa*).	May 9 to July 25__	97	246.8	109.1	155.0	44	1421	628
		98	241.0	97.5	153.2	40	1571	636
		99	251.2	102.3	158.0	41	1545	629
		100	256.8	99.6	157.2	39	1578	612
		101	265.0	112.6	157.5	42	1399	594
		102	244.7	104.8	144.8	43	1382	592
Mean___							1483 ± 31	615 ± 6
Rye, spring, C. I. 73 (*Secale cereale*).	May 9 to July 25__	103	182.1	53.7	116.4	29	2168	639
		104	193.5	56.5	121.6	29	2152	628
		105	203.2	56.0	119.1	28	2127	586
		106	173.1	42.6	110.9	25	2603	641
		107	206.8	57.0	131.5	28	2307	636
		108	212.4	53.5	127.9	25	2391	602
Mean___							2291 ± 54	622 ± 7

WATER REQUIREMENT OF CORN, SORGHUM, AND MILLET, 1914

Nine varieties and five hybrids of corn were grown in 1914: The water requirements based on dry matter production, was:

Corn:

Tom Thumb	315 ± 8
Algeria	330 ± 4
Budapest	345 ± 3
China White	344 ± 7
Pima	365 ± 7
Northwestern Dent	368 ± 6
Joaquin	368 ± 9
German C24–2	372 ± 3
German C24–1	385 ± 5
Average	355 ± 6

Hybrids:

Algeria × China	347 ± 5
Joaquin × Budapest	365 ± 5
German C24–1 × German C24–2	372 ± 1
Budapest × Pima	388 ± 5
Joaquin × Pima	389 ± 9
Average	372 ± 5
Average for series	361 ± 6

Of the corn varieties, Tom Thumb is the lowest in water-requirement value. It was grown during the late season (Table 7). The highest water-requirement value is shown by a German sweet corn, C24–1. A comparison of the hybrids and their parents shows that the hybrids range in water requirement from 10 per cent below to 10 per cent above the parental mean. The chances are even also that a maize hybrid will not depart in its water requirement more than ± 6 per cent from the parental mean.[10]

The water requirement of the hybrids was 3 per cent higher than that of the corn group as a whole, which was considerably lowered by the inclusion of Tom Thumb, a variety not used in hybridization. It is interesting to compare these corn varieties on the basis of their water requirement. Tom Thumb, which stands first in the line of efficiency, is a very diminutive corn, so small that is has little or no practical value. Next in efficiency are Algeria, Budapest, China White, Pima, and Northwestern Dent, the latter a short season crop grown in the northern Plains region.

Only two sorghum varieties were grown in 1914, Dakota Amber and Minnesota Amber. Minnesota Amber has a slightly lower water requirement than Dakota Amber, their values being 284 ± 3 and 296 ± 1, respectively (Table 4). The probable error in the determination of water requirement of sorghum is usually about 1 per cent. The difference in water requirement of these two varieties is approximately 4 per cent. Sorghum was 19 per cent more efficient in the use of water than the corn varieties. Even in grain production these forage sorghums have a low water requirement, the value being 893 ± 26 for Minnesota Amber and 898 ± 50 for Dakota Amber, or 76 per cent of the water requirement for barley, the most efficient of the small grains.

Kursk millet, with a water requirement of 295 ± 2, is much more efficient than Siberian millet, with a water requirement of 316 ± 5. The millets in this case, have a water requirement 5 per cent higher than the sorghums, but 15 per cent lower than corn. On the basis of grain production, Kursk has a water requirement of 1075 ± 38 and Siberian of 1162 ± 51. In other words it is about as efficient as barley. Kursk millet which was also grown as a late season crop (see Table 7), gave a value 4 per cent lower than when grown in the spring.

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[10] BRIGGS, L. J., and SHANTZ, H. L. Op. cit. 1915. p. 401.

WATER REQUIREMENT OF LEGUMES, 1914

Fourteen varieties of legumes were grown in the water requirement experiments in 1914. The water requirements of these varieties, based on dry matter, was:

Crimson clover	517 ± 19	Alfalfa, A. D. I. 162–98	904 ± 12
Hairy vetch	531 ± 3	Alfalfa, A. D. I. E–23–20–52	906 ± 12
Guar	544 ± 8	Alfalfa, A. D. I. 162–98–B	906 ± 12
Vetch, black bitter	584 ± 12	Alfalfa, A. D. I. E–5–30	933 ± 11
Cowpea	659 ± 5	Alfalfa, Grimm, A. D. I. H–	
Alfalfa, A. D. I. 162–98–A	810 ± 5	4–60	957 ± 8
Alfalfa, Hansen	846 ± 27		
Lupinus albus	870 ± 34	Average	776 ± 15
Alfalfa, Grimm, A. D. I. E–23	890 ± 6		

TABLE 4—*Water requirement based on dry matter, of corn, sorghum, millet, and sudan grass, at Akron, Colo, 1914*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
CORN								
(*Zea mays*)			*Grams*	*Grams*	*Kgms.*	*P. ct*		
Algeria×China	June 3 to Aug. 31	243	515.0		176.3			342
		244	474.2		175.0			369
		245	524.4		178.3			340
		246	573.0		187.6			327
		247	477.5		170.9			358
Mean								347±5
China White	June 3 to Aug. 31	248	401.3		149.2			372
		249	313.9		104.2			332
		250	394.7		141.1			357
		251	406.7		130.1			320
		252	419.3		143.1			341
Mean								344±7
Joaquin	June 3 to Aug. 31	253	343.3		140.7			410
		254	287.0		91.3			318
		255	96.3		35.1			364
		256	201.7		74.7			370
		257	301.0		113.6			377
Mean								368±9
Joaquin×Budapest	June 3 to Aug. 31	258	410.3		142.4			347
		259	453.5		159.4			351
		260	382.7		148.9			389
		261	358.0		131.8			368
		262	388.4		143.4			369
Mean								365±5
Budapest	June 3 to Sept. 1	263	427.5		150.2			351
		264	398.3		134.4			337
		265	451.7		150.8			334
		266	379.7		133.2			351
		267	396.7		139.3			351
Mean								345±3
Budapest×Pima	June 3 to Sept. 1	268	368.4		137.4			373
		269	364.8		149.8			411
		270	362.6		144.1			397
		271	364.2		138.4			380
		272	348.5		132.8			381
Mean								388±5

TABLE 4.—*Water requirement based on dry matter, of corn, sorghum, millet, and sudan grass, at Akron, Colo., 1914*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on— Grain	Water requirements based on— Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct*		
CORN—continued								
(*Zea Mays*)								
Pima	June 3 to Sept. 1	273	350. 7		134. 0			382
		274	343. 0		132. 0			385
		275	375. 3		123. 2			328
		276	335. 2		120. 5			359
		277	402. 3		149. 6			372
Mean								365±7
Joaquin×Pima	June 3 to Sept. 1	278	365. 5		151. 4			414
		279	388. 9		161. 2			415
		280	352. 3		126. 8			360
		281	324. 9		121. 3			373
		282	433. 3		165. 2			381
Mean								389±9
Northwestern Dent	June 3 to Aug. 31	217	308 3		119. 2			387
		218	281. 2		108. 1			384
		219	298 1		109. 1			366
		220	269. 2		102. 0			379
		221	300. 1		109. 0			363
		222	369. 2		121. 8			330
Mean								368±6
German C24–1	June 3 to Sept. 1	223	354. 4		135. 4			382
		224	296. 7		107. 5			362
		225	333. 2		129. 8			390
		226	306. 4		122. 4			399
		227	317. 4		125. 1			394
Mean								385±5
German C24–1 and C24–2	June 3 to Sept. 1	228	419. 0		155. 6			371
		229	432. 4		158. 2			366
		230	362. 2		136. 3			376
		231	357. 0		133. 7			375
		232	379. 5		140. 4			370
Mean								372±1
German C24–2	June 3 to Aug. 31	233	374. 6		143. 8			384
		234	407. 5		148. 2			364
		235	399. 8		150. 0			375
		236	379. 7		139. 9			368
		237	335. 0		123. 2			368
Mean								372±3
Algeria	June 3 to Aug. 31	238	354. 7		117. 8			332
		239	521. 2		161. 1			309
		240	431. 3		149. 0			345
		241	392. 0		131. 4			335
		242	386. 7		127. 8			330
Mean								330±4
SORGHUM								
(*Andropogon sorghum*)								
Minnesota, Black Amber, A. D. I. 341–13.	June 9 to Sept. 1	205	538. 7	191. 9	161. 3	36	841	299
		206	434. 8	132. 8	128. 2	31	965	295
		207	443. 4	141. 9	123. 9	32	873	279
		208	478. 5	172. 5	133. 7	36	775	279
		209	403. 0	110. 1	113. 0	27	1026	280
		210	444. 2	136. 8	119. 7	31	875	269
Mean							893±26	284±3

TABLE 4.—*Water requirement based on dry matter, of corn, sorghum, millet, and sudan grass, at Akron, Colo., 1914*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
SORGHUM—continued								
(*Andropogon sorgham*)			*Grams*	*Grams*	*Kgms.*	*P. ct*		
Dakota Amber, A. D. l. 341-10-4.	June 9 to Sept. 1	211	476.0	139.6	137.5	29	985	289
		212	430.0	170.8	128.8	40	754	300
		213	399.6	147.1	117.9	37	801	295
		214	401.1	139.5	120.6	35	865	301
		215	433.6	167.0	128.7	39	771	297
		216	419.7	101.4	122.5	24	1208	292
Mean							898±50	296±1
SUDAN GRASS, S. P. I 25017								
(*Andropogon sorghum aethiopicus*)								
First crop	June 10 to Aug. 12	410	353.5		129.2			365
		411	244.9		88.1			360
		412	363.0		127.6			352
		413	270.1		102.1			378
		414	275.9		103.0			373
Mean								366±3
Second crop	Aug. 13 to Oct. 15	410	64.5		35.0			543
		411	58.2		29.2			502
		412	63.3		34.6			547
		413	55.9		30.9			553
		414	51.0		28.2			553
Mean								540±6
Combined crops	June 10 to Oct. 15	410	418.0		164.2			393
		411	303.1		117.3			387
		412	426.3		162.2			380
		413	326.0		133.0			408
		414	326.9		131.2			401
Mean								394±4
MILLET								
(*Chaetochloa italica*)								
Kursk, S. P. I. 34771	June 9 to Aug. 12	181	322.1	80.2	92.8	25	1157	288
		182	274.8	88.6	81.6	32	921	297
		183	276.8	82.1	80.2	30	977	290
		184	303.6	70.4	91.3	23	1297	301
		185	311.2	85.9	91.4	28	1064	294
		186	318.7	92.4	95.4	29	1032	299
Mean							1075±38	295±2
Siberian, A. D. I 3-4	June 9 to Aug. 12	187	325.2	104.2	98.7	32	947	304
		188	321.1	91.2	106.4	28	1167	331
		189	323.2	91.6	92.3	28	1008	286
		190	295.1	66.8	92.7	23	1388	314
		191	293.6	88.4	99.2	30	1122	338
		192	348.6	83.4	111.6	24	1338	320
Mean							1162±51	316±5

The first four legume crops were grown during the late season and are therefore not strictly comparable. However, this is true of all crops that do not have the same length of growth period. The results obtained with these four crops are therefore included with the other measurements in the summary tables. The legumes at Akron range in their water requirement from crimson clover at 517 ± 19, the

lowest, to a Grimm alfalfa at 957 ± 8, the highest, a difference of 85 per cent. Legumes usually have a high water requirement but some of the varieties, crimson clover, hairy vetch, guar, and bitter vetch each have a water requirement of less than 600, while the Grimm alfalfas average about 900. Of the alfalfas, *Medicago falcata* has a water requirement 7 per cent lower than the average of the Grimm varieties, but not lower than the range of the latter, since one of the Grimm varieties, A. D. I. 162-98-A, showed a water requirement 4 per cent lower than *M. falcata*. (See Table 5.)

TABLE 5.—*Water requirement, based on dry matter, of legumes, at Akron, Colo., 1914*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
ALFALFA								
(*Medicago sativa*)			*Grams*	*Grams*	*Kgms.*	*P ct*		
Grimm, A. D. I. E23–20–52, first crop.	June 5 to July 11__	109	70. 3	_____	43. 4	_____	_____	617
		110	77. 9	_____	49. 3	_____	_____	633
		111	78. 6	_____	47. 6	_____	_____	606
		112	79. 3	_____	51. 1	_____	_____	644
		113	54. 5	_____	33. 5	_____	_____	615
		114	72. 0	_____	43. 1	_____	_____	599
Mean_____								619±5
Grimm, second crop_____	July 12 to Aug. 16__	109	56. 4	_____	57. 0	_____	_____	1011
		110	69. 9	_____	65. 7	_____	_____	940
		111	77. 5	_____	68. 3	_____	_____	881
		112	83. 4	_____	72. 8	_____	_____	873
		113	50. 5	_____	39. 5	_____	_____	782
		114	60. 2	_____	55. 0	_____	_____	914
Mean_____								900±21
Grimm, third crop_____	Aug. 16 toOct. 26__	109	137. 8	_____	146. 2	_____	_____	1061
		110	120. 0	_____	136. 9	_____	_____	1141
		111	142. 7	_____	144. 1	_____	_____	1010
		112	132. 0	_____	137. 1	_____	_____	1039
		113	86. 0	_____	93. 4	_____	_____	1086
		114	101. 3	_____	120. 6	_____	_____	1191
Mean_____								1088±20
Grimm, combined crop_____	June 5 to Oct. 26__	109	264. 5	_____	246. 6	_____	_____	932
		110	267. 8	_____	251. 9	_____	_____	941
		111	298. 8	_____	260. 0	_____	_____	870
		112	294. 7	_____	261. 0	_____	_____	886
		113	191. 0	_____	166. 4	_____	_____	871
		114	233. 5	_____	218. 7	_____	_____	937
Mean_____								906±12
Grimm, A. D. I. E23, first crop.	June 5 to July 11__	115	61. 6	_____	38. 9	_____	_____	631
		116	94. 7	_____	56. 7	_____	_____	599
		117	87. 3	_____	53. 0	_____	_____	607
		118	94. 1	_____	57. 2	_____	_____	⁍608
		119	87. 7	_____	53. 6	_____	_____	611
		120	98. 2	_____	59. 2	_____	_____	603
Mean_____								610±3
Grimm, second crop_____	July 12 to Aug. 15_	115	68. 6	_____	60. 3	_____	_____	879
		116	85. 7	_____	75. 1	_____	_____	876
		117	70. 6	_____	67. 7	_____	_____	959
		118	74. 1	_____	66 3	_____	_____	895
		119	71. 0	_____	68. 3	_____	_____	962
		120	91. 7	_____	79. 5	_____	_____	867
Mean_____								906±14

TABLE 5.—*Water requirement, based on dry matter, of legumes, at Akron, Colo., 1914*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
ALFALFA—continued			*Grams*	*Grams*	*Kgms.*	*P. ct*		
(*Medicago sativa*)								
Grimm, third crop	Aug. 16 to Oct. 26	115	111. 5		126. 4			1134
		116	154. 0		159. 3			1034
		117	130. 6		137. 4			1052
		118	139. 0		148. 6			1069
		119	142. 3		142. 9			1004
		120	172. 5		179. 3			1039
Mean								1055±12
Grimm, combined crop	June 5 to Oct. 26	115	241. 7		225. 6			933
		116	334. 4		291. 1			871
		117	288. 5		258. 1			895
		118	307. 2		272. 1			886
		119	301. 0		264. 8			880
		120	362. 4		318. 0			877
Mean								890±6
Grimm, A. D. I. F-5-30, first crop.	June 5 to July 11	121	87. 0		59. 1			679
		122	83. 8		52. 4			625
		123	86. 2		53. 8			624
		124	79. 0		52. 8			668
		125	94. 0		59. 4			632
		126	81. 4		54. 0			663
Mean								649±8
Grimm, second crop	July 12 to Aug. 15	121	73. 9		72. 0			974
		122	75. 3		68. 9			915
		123	74. 5		70. 2			942
		124	73. 1		68. 2			933
		125	83. 8		71. 7			856
		126	71. 5		67. 6			945
Mean								928±11
Grimm, third crop	Aug. 16 to Oct. 26	121	126. 6		158. 9			1255
		122	127. 6		139. 4			1092
		123	122. 5		131. 4			1073
		124	121. 7		137. 8			1132
		125	128. 4		143. 7			1119
		126	117. 6		130. 7			1111
Mean								1130±16
Grimm, combined crop	June 5 to Oct. 26	121	287. 5		290. 0			1009
		122	286. 7		260. 7			909
		123	283. 2		255. 4			902
		124	273. 8		258. 8			945
		125	306. 2		274. 8			897
		126	270. 5		252. 3			933
Mean								933±11
A. D. I. 162-98-A, first crop.	May 9 to July 11	127	229. 0		159. 6			697
		128	203. 1		147. 1			724
		129	202. 6		142. 9			705
		130	172. 7		127. 0			735
		131	208. 4		150. 7			723
		132	201. 9		148. 7			737
Mean								720±5
A. D. I. 162-98-A, second crop.	July 12 to Aug. 15	127	146. 3		124. 6			852
		128	139. 1		118. 7			853
		129	135. 9		103. 0			758
		130	135. 4		106. 3			785
		131	142. 9		110. 7			775
		132	175. 4		145. 5			830
Mean								809±14

TABLE 5.—*Water requirement, based on dry matter, of legumes, at Akron, Colo., 1914*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
ALFALFA—continued								
(*Medicago salina*)			*Grams*	*Grams*	*Kgms.*	*P. ct*		
A. D. I. 162-98-A, third crop	Aug. 16 to Oct. 26	127	208. 5		187. 2			898
		128	205. 6		186. 5			907
		129	206 4		183. 9			891
		130	200. 7		182. 2			908
		131	214. 3		188 9			881
		132	216. 5		196. 1			906
Mean								899±3
A. D I. 162-98-A, combined crop	May 9 to Oct 26	127	583. 8		471. 4			807
		128	547. 8		452 3			828
		129	544. 9		429. 8			789
		130	508 8		415 5			817
		131	565 6		450. 3			796
		132	593. 8		490. 3			828
Mean								810±5
A. D. J. 162-98, first crop	June 5 to July 11	133	91. 5		60 7			663
		134	90. 0		56. 2			624
		135	73. 2		42. 6			582
		136	74. 8		42. 6			570
		137	81. 3		49. 7			611
		138	69 0		39. 7			575
Mean								604±11
A. D. J. 162-98, second crop	July 12 to Aug. 15	133	65. 3		62 6			959
		134	72. 3		61. 6			852
		135	61. 3		57. 0			930
		136	67. 9		58 2			857
		137	69. 0		61 3			888
		138	66. 8		56. 8			850
Mean								889±14
A. D. I 162-98, third crop	Aug. 16 to Oct. 26	133	119 6		147 2			1231
		134	139. 4		146. 8			1053
		135	87. 6		101. 3			1156
		136	119. 4		131. 5			1101
		137	110. 0		130. 1			1183
		138	99. 8		103. 8			1040
Mean								1127±24
A. D. I. 162-98, combined crop	June 5 to Oct. 26	133	276. 4		270. 5			979
		134	301. 7		264. 6			877
		135	222. 1		200. 9			905
		136	262. 1		232. 3			886
		137	260 3		241. 1			926
		138	235. 6		200. 3			850
Mean								904±12
A. D. I. 162-98-B, first crop	May 9 to July 11	139	178. 9		126. 2		●	705
		140	166. 2		128. 4			773
		141	153. 2		114. 0			744
		142	155. 8		123. 0			789
		143	167. 7		127. 7			761
		144	175. 2		138 3			789
Mean								760±9
A. D. I. 162-98-B, second crop	July 12 to Aug. 15	139	125. 0		101. 1			809
		140	116. 6		97. 7			838
		141	107. 8		90. 0			835
		142	112. 5		97. 6			868
		143	122. 4		102. 8			840
		144	134. 7		114. 1			847
Mean								840±5

TABLE 5.—*Water requirement, based on dry matter, of legumes, at Akron, Colo.,* *1914*—Continued

Kind of plant	Period of growth	Pot No	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
ALFALFA—continued								
(*Medicago sativa*)			*Grams*	*Grams*	*Kgms.*	*P ct*		
A. D. I. 162-98-B, third crop.	Aug. 16 to Oct. 26	139	183. 5	-------	184 4	-----	---------	1005
		140	143. 3	-------	173. 6	-----	---------	1211
		141	182. 2	-------	185 3	-----	---------	1017
		142	196. 3	-------	198 0	-----	---------	1009
		143	146. 0	-------	174. 9	-----	---------	1198
		144	206. 8	-------	233. 9	-----	---------	1131
Mean								1095±32
A. D. I 162-98-B, combined crop.	May 9 to Oct. 26	139	487. 4	-------	411. 7	-----	---------	845
		140	426. 1	-------	399. 7	-----	---------	938
		141	443. 2	-------	389 3	-----	---------	878
		142	464. 6	-------	418. 6	-----	---------	901
		143	436. 1	-------	405. 4	-----	---------	930
		144	516. 7	-------	486 3	-----	---------	941
Mean								906±12
Grimm A. D. I H-4-60, first crop.	June 5 to July 11	151	95. 0	-------	62. 4	-----	---------	657
		152	96. 9	-------	62. 1	-----	---------	641
		153	96. 3	-------	63. 4	-----	---------	658
		154	99. 2	-------	68. 6	-----	---------	692
		155	97. 2	-------	65. 5	-----	---------	674
		156	88. 4	-------	55. 5	-----	---------	628
Mean								658±6
A. D. I. H-4-60, second crop.	July 12 to Aug. 15	151	86. 0	-------	79. 0	-----	---------	919
		152	84. 4	-------	75. 5	-----	---------	895
		153	78. 1	-------	73. 0	-----	---------	935
		154	86. 0	-------	78. 1	-----	---------	908
		155	70 6	-------	67. 9	-----	---------	962
		156	73. 2	-------	67. 6	-----	---------	923
Mean								924±6
A. D. I. H-4-60, third crop	Aug. 16 to Oct. 26	151	107. 9	-------	147. 5	-----	---------	1367
		152	119. 8	-------	140. 9	-----	---------	1176
		153	113 6	-------	141. 6	-----	---------	1246
		154	108. 6	-------	138. 0	-----	---------	1271
		155	116 0	-------	132. 8	-----	---------	1145
		156	105. 8	-------	128. 9	-----	---------	1218
Mean								1237±22
A. D. I. H-4-60, combined crop.	June 5 to Oct. 26	151	288. 9	-------	288. 9	-----	---------	1000
		152	301. 1	-------	278. 5	-----	---------	925`
		153	288. 0	-------	278. 0	-----	---------	965
		154	293. 8	-------	284. 7	-----	---------	969
		155	283 8	-------	266. 2	-----	---------	938
		156	267. 4	-------	252. 0	-----	---------	942
Mean								957±8
(*Medicago falcata*)								
Hansen seed, first crop	June 9 to July 11	145	25. 2	-------	16 6	-----	---------	659
		146	23 9	-------	16. 9	-----	---------	707
		147	21. 9	-------	12. 0	-----	---------	548
		148	16. 0	-------	11. 4	-----	---------	713
		149	28. 0	-------	18. 0	-----	---------	643
		150	19. 5	-------	12 5	-----	---------	641
Mean								652±16
Hansen seed, second crop	July 12 to Aug. 15	145	52. 9	-------	42. 4	-----	---------	802
		146	49. 0	-------	38. 5	-----	---------	786
		147	15. 2	-------	10. 6	-----	---------	697
		148	46. 8	-------	34. 2	-----	---------	731
		149	41. 0	-------	32. 2	-----	---------	785
		150	33. 3	-------	23. 6	-----	---------	709
Mean								752±15

TABLE 5.—*Water requirement, based on dry matter, of legumes, at Akron, Colo., 1914*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
ALFALFA—continued								
(*Medicago falcata*)			*Grams*	*Grams*	*Kgms.*	*P. ct.*		
Hansen seed, third crop	Aug. 16 to Oct. 26	145	29.9		36.4			1217
		146	22.4		31.6			1411
		147	39.4		29.4			746
		148	25.0		33.3			1332
		149	22.4		32.3			1442
		150	23.8		25.2			1059
Mean								1201±75
Hansen seed, combined crop	June 9 to Oct. 26	145	108.0		95.4			883
		146	95.3		87.0			913
		147	76.5		52.0			680
		148	87.8		78.9			899
		149	91.4		82.5			903
		150	76.6		61.3			800
Mean								846±27
COWPEA								
(*Vigna sinensis*)								
S. P. I. 29282	May 28 to Aug. 12	169	179.6	63.4	117.5	35	1853	654
		170	169.7	52.7	108.6	31	2061	640
		171	161.7	56.2	106.9	35	1902	661
		172	163.3	55.8	110.6	34	1982	677
		173	171.9	57.8	110.5	34	1912	643
		174	175.4	57.9	119.3	33	2060	680
Mean							1962±27	659±5
Lupinus albus								
S. P. I. 35477	May 28 to Aug. 12	175	84.1	11.6	82.2	14	7086	977
		176	64.6	10.5	58.7	16	5590	909
		177	116.9	24.0	95.0	21	3958	813
		178	100.4	19.1	82.1	19	4298	818
		179	117.7	22.6	83.2	19	3681	707
		180	54.7	1.4	54.4	(a)	(a)	995
Mean							4923±478	870±34

[a] Omitted in computing mean.

Cowpeas, an important field crop, require 659 ± 5, or 28 per cent, less water than the Grimm varieties of alfalfa, but more than twice as much as sorghum. *Lupinus albus* requires 870 ± 34, or almost as much as alfalfa. On the basis of seed production, cowpeas gave a water requirement of 1962 ± 27 and *L. albus* 4923 ± 478.

WATER REQUIREMENT OF COTTON, PIGWEED, AND GRAMA GRASS, 1914

Cotton was included in the experiments each year at Akron. Notwithstanding the fact that cotton was far from its natural range the water requirement of that crop, 574 ± 9, was as low as for oats and almost as low as for wheat (Table 6).

Pigweed produced three crops, the combined crop having a water requirement of 306 ± 1, and was exceeded only by millet and sorghum in the efficient use of water. Pigweed was about three times as efficient as alfalfa and twice as efficient as oats and some of the wheats.

TABLE 6.—*Water requirement, based on dry matter, of cotton, pigweed, and grama grass, at Akron, Colo., 1914*

Kind of plant	Period of growth	Pot No.	Dry matter	Water	Water requirements based on dry matter
COTTON					
(*Gossypium hirsutum*)			*Grams*	*Kgms.*	
Triumph	June 17 to Oct. 15	193	363.8	194.6	535
		194	345.1	194.1	562
		195	360.8	199.1	552
		196	328.5	190.2	579
		197	311.7	187.5	602
		198	370.7	226.6	611
Mean					574±9
PIGWEED					
(*Amaranthus retroflexus*)					
First crop	June 3 to July 14	163	124.0	39.9	322
		164	115.8	38.2	330
		165	127.0	37.9	298
		166	118.7	36.3	306
		167	126.7	40.5	320
		168	131.0	39.8	304
Mean					313±4
Second crop	July 15 to Aug. 13	163	57.8	17.6	304
		164	55.2	15.9	288
		165	54.1	16.2	299
		166	49.4	15.8	320
		167	48.6	14.5	298
		168	57.8	18.0	311
Mean					303±3
Third crop	Aug. 14 to Oct 17	163	44.7	12.0	268
		164	44.0	12.3	280
		165	44.1	13.7	311
		166	36.8	10.8	293
		167	41.6	11.9	286
		168	42.3	12.0	284
Mean					287±4
Combined crop	June 3 to Oct. 17	163	226.5	69.5	307
		164	215.0	66.4	309
		165	225.2	67.8	301
		166	204.9	62.9	307
		167	216.9	66.9	308
		168	231.1	69.8	302
Mean					306±1
GRAMA GRASS					
(*Bouteloua gracilis*)					
First crop	June 3 to July 14	157	26.6	9.8	368
		158	23.1	7.9	342
		159	28.1	9.1	324
		160	28.5	9.6	337
		161	27.9	8.8	315
		162	24.7	7.1	287
Mean					329±8
Second crop	July 15 to Aug. 12	157	36.2	11.7	323
		158	28.4	9.9	349
		159	29.7	10.4	350
		160	35.6	10.8	303
		161	24.9	8.9	357
		162	28.0	9.4	336
Mean					336±6

TABLE 6.—*Water requirement, based on dry matter, of cotton, pigweed, and grama grass, at Akron, Colo.; 1914*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Water	Water requirements based on dry matter
GRAMA GRASS—continued					
(*Boutelaua gracilis*)			*Grams*	*Kgms.*	
Third crop	Aug. 13 to Oct 16	157	11. 6	10. 7	922
		158	13. 7	8. 3	606
		159	19 5	10. 2	523
		160	19. 8	9. 6	485
		161	14 9	8 4	564
		162	13. 0	7. 8	600
Mean					617±39
Combined crop	June 3 to Oct. 16	157	74. 4	32. 2	433
		158	65 2	26. 1	400
		159	77. 3	29. 7	384
		160	83 9	30 0	358
		161	67. 7	26. 1	386
		162	65. 7	24 3	370
Mean					389±7

Three crops of grama grass gave a combined water requirement, based on dry matter, of 389 ± 7. This is the most important of the native grasses. It is somewhat less efficient than corn in the use of water, and requires 34 per cent more water than the sorghum varieties tested.

WATER REQUIREMENT OF PLANTS GROWN DURING LATE SUMMER AND AUTUMN, 1914

Thirteen late-season crops were grown, the time of planting ranging from August 28 to September 4. Grouped in the order of their efficiency in the use of water for dry matter production these crops are as follows:

Tumbleweed	272 ± 4	Guar	544 ± 8
Kursk millet	284 ± 4	Buffalo bur	557 ± 7
Tom Thumb corn	315 ± 8	Bitter vetch	584 ± 12
Clammyweed	502 ± 11	Polygonum	705 ± 50
Nightshade	506 ± 14	Verbena	730 ± 20
Crimson clover	517 ± 19	Franseria	1176 ± 47
Hairy vetch	531 ± 3		

It will be seen that the same variety of millet, Kursk, S. P. I. 34771, was approximately 4 per cent more efficient when grown as a late-season crop than when grown during the regular season.

The results obtained in these measurements as shown in Table 7 are included in the records obtained from those made earlier in the season, since there is a close agreement between the water requirement values of plants grown late in the season and those of the same plants grown early in the season. Many of the plants are weeds and thrive naturally during the late summer and autumn. There is a surprisingly wide range in water requirement among the common weeds. Tumbleweed is more efficient than millet or Tom Thumb corn. The legumes have a relatively low water requirement. The

water requirement values of erimson clover, hairy vetch, guar, and bitter vetch are aproximately the same as the wheats, while polygonum and verbena, roadside weeds, require more water than any crop group except alfalfa. Franseria, a native plant of wet bottoms, showed a water requirement higher than any of the series.

TABLE 7.—*Water requirement, based on dry matter, of plants grown during late summer and autumn at Akron, Colo., 1914*

Kind of plant	Period of growth	Pot No.	Dry matter	Water	Water requirements based on dry matter
			Grams	*Kgms.*	
Clammyweed (*Polanisia*) following Baluchistan wheat.	Aug. 29 to Oct. 17	37	4. 8	2 5	521
		38	6. 1	3. 2	525
		39	6. 3	3. 3	524
		40	6 8	3. 2	471
		42	4. 5	2. 1	467
Mean					502±11
Millet, Kursk (*Chaetochloa italica*), following Siberia wheat.	Aug 28 to Oct. 15	49	31. 2	8. 2	263
		50	50. 1	15. 0	299
		51	69. 5	20. 5	295
		52	61. 7	17. 6	285
		53	69. 0	18. 9	274
		54	44. 8	12. 8	286
Mean					284±4
Tumbleweed (*Amaranthus graecizans*), following Peru wheat.	Aug 28 to Oct. 13	55	49. 9	12. 4	248
		56	46. 5	13. 1	282
		57	45 1	12. 5	277
		58	47. 0	13. 3	283
		59	36. 2	9. 5	262
		60	56. 4	15. 9	282
Mean					272±4
Corn, Tom Thumb (*Zea mays*), following Canada (Marquis) wheat.	Aug. 28 to Oct. 13	61	51. 1	16. 0	313
		62	63. 2	16. 7	264
		63	61. 2	19. 3	315
		64	42. 1	15. 5	368
		65	65. 0	19. 6	302
		66	64. 5	21 3	330
Mean					315±8
Guar, following Jumillo wheat	Aug. 28 to Oct. 12	67	6. 9	3. 6	522
		68	6. 7	3. 7	552
		69	7. 2	4. 3	597
		70	3. 7	2. 0	541
		71	9. 6	5. 2	542
		72	8. 4	4. 3	512
Mean					544±8
Crimson clover, following Jumillo wheat × Velvetchaff wheat (*Trifolium incarnatum*).	Sept. 4 to Oct 27	73	22. 4	10. 5	469
		74	16. 7	7. 8	467
		75	17. 1	7. 9	462
		76	14. 8	7. 7	520
		77	23. 7	13. 2	557
		78	26. 4	16 6	629
Mean					517±19
Black bitter vetch (*Vicia ervillea*) following Velvet chaff wheat	Sept. 4 to Oct. 27	79	17. 9	9. 9	553
		80	39. 3	21. 7	552
		81	40. 7	26. 3	646
		82	32. 0	19. 3	603
		83	26. 6	14. 8	556
		84	24. 2	14. 4	595
Mean					584±12

TABLE 7.—*Water requirement, based on dry matter, of plants grown during late summer and autumn at Akron, Colo., 1914*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Water	Water requirements based on dry matter
			Grams	*Kgms.*	
Polygonum ariculare, following Hannchen barley.	Aug. 28 to Oct. 27	85	10.3	7.7	748
		86	3.2	1.6	500
		87	5.1	4.0	784
		88	7.6	6.0	789
Mean					705±50
Buffalo bur (*Solanum rostratum*), following Swedish Select oats.	Aug. 28 to Oct 15	91	51.3	29.7	579
		92	51.9	29.0	559
		93	51.8	29.3	566
		94	56.0	29.5	527
		95	49.5	28.5	576
		96	55.3	29.6	535
Mean					557±7
Nighshade (*Solanum triflorum*), following Burt oats.	Aug 29 to Oct. 27	97	73.3	41 2	562
		98	68.6	38.7	564
		99	59.6	29.5	495
		100	57.5	28 1	489
		101	62.8	27.3	435
		102	62.7	30.9	493
Mean					506±14
Verbena (*Verbena bractiosa*), following Vern rye.	Aug. 29 to Oct. 27	103	20.3	14.4	709
		104	24 0	15.4	642
		105	20.9	14 5	694
		106	20.7	14.9	720
		107	13.9	10.3	741
		108	13.5	11.8	874
Mean					730±20
Franseria tenuifolia, following cowpea	Aug 29 to Oct. 27	169	4.8	4.7	979
		170	7.7	8 2	1065
		171	11.3	15 1	1336
		172	14.6	19.9	1363
		173	11.5	12.2	1061
		174	13 6	17.0	1250
Mean					1176±53
Hairy vetch (*Vicia villosa*), following *Lupinus albus*.	Sept. 4 to Oct. 27	175	36.4	19.2	527
		176	43.5	22.5	517
		177	26.5	14.3	540
		178	27 9	15.2	545
		179	31.0	16.5	532
		180	27.3	14.3	524
Mean					531±3

SUMMARY OF WATER-REQUIREMENT MEASUREMENTS MADE IN 1914

In order to compare rapidly the water requirement of the various crops for the different years and to eliminate errors due to averaging plants of low and high water requirement, which would be equivalent to weighting those of high-water requirement, the yearly values have been expressed as proportions of the average for the period of years during which the crop was grown, which value was fixed at 100. This gives a truer value for each year than could be obtained by averaging actual values. In the discussion the values thus obtained have been treated as index numbers and the expression "points above" or "points below" have been used to express the relative positions. To express these differences in percentages would lead to confusion.

It is the opinion of the writers that the facts are well shown by this simple method of treatment which is here followed in order to avoid the use of logarithms in the discussion of the data. The procedure will be clearly understood by comparing Table 27, the actual values, with Table 28, the index values.

The climatic conditions controlling the use of water in the 1914 experiments were near the average for the period 1911–1917. (See Tables 27 and 28.) As compared with the previous years the water requirement was about the same as in 1913, apparently a little less than 1911, and about 25 points higher than in 1912. The latter year was unusually favorable. On the whole the weather was cool and relatively damp and the sun's intensity was about 20 per cent below normal.[11]

The water requirement in 1914 was 26 points higher than the water requirement in the cool, damp year, 1915; 2 points higher than in 1917, which was nearly an average year; and 28 points lower than in the dry year, 1916.

During 1914 tumbleweed showed the greatest efficiency in the use of water, its water-requirement value being 272 ± 4. It was followed closely by Minnesota Amber and Dakota Amber sorghum, with values of 284 ± 3 and 296 ± 1, respectively, and Kursk millet with a value of 295 ± 2. The corn varieties ranged in water-requirement value from 315 ± 8 to 389 ± 9. The range in values of other crops were as follows: Wheats, from 458 ± 10 to 689 ± 8; barley, 501 ± 5; oats from 599 ± 2 to 615 ± 6; rye, 622 ± 7; legumes from 517 ± 19 for crimson clover to 957 ± 8 for a Grimm alfalfa; while weeds ranged from 272 ± 4 for tumbleweed to 1176 ± 53 for Franseria. As a general statement, the sorghums, millets, and corns were the most efficient of the crop plants in the use of water, small grains were intermediate, and legumes were least efficient.

On the basis of grain production, sorghums ranging from 893 ± 26 to 898 ± 50 were most efficient in the use of water; millets ranging from 1075 ± 38 to 1162 ± 51 were next; followed by the small grains, ranging from 1179 ± 28 for Hannchen barley, to 3564 ± 98 for Pacific Bluestem wheat.

Based on seed production the legumes showed very high water requirement. Cowpeas gave a value of 1962 ± 27 and *Lupinus albus* of 4923 ± 478.

WATER-REQUIREMENT EXPERIMENTS, 1915

In 1915, 67 sets of plants were grown, the results from 39 of which are included in the relative water-requirement figures here presented. The year was unusually cool and damp, and the water requirement, the evaporation, and the temperature were lower than during any other year of the experimental period under discussion. (See Tables 27, 28, 31, and 32.) Special attention was given during 1915 to wheat and flax.

WATER REQUIREMENT OF VARIETIES OF WHEAT, 1915

Eighteen varieties of wheat were grown at Akron in 1915. Eight of these were durum wheats, 9 were common varieties, and 1 was a hybrid. The results, based on dry matter, are as follows:

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[11] Briggs, L. J., and Shantz, H. L. Op. cit. 1914. p. 54.

Durum wheats:
    C. I. 4131 (old seed) _____ 357 ± 4
    C. I. 4131 _____ 364 ± 6
    Beloturka, C. I. 3705 (old
      seed)_____ 387 ± 3
    Beloturka, C. I. 3705_____ 390 ± 2
    Jumillo, C. I. 1736_____ 396 ± 6
    Kubanka, C. I. 1440_____ 405 ± 3
    C. I. 4082 (old seed) _____ 413 ± 6
    C. I. 4082_____ 431 ± 3

    Average_____ 393 ± 4

Common wheats:
    Marquis, C. I. 3641 (old
      seed)_____ 412 ± 3
    Marquis, C. I. 3641_____ 424 ± 3

Common wheat—Continued.
    Preston, C. I. 3328_____ 452 ± 6
    C. I. 4090 (old seed) _____ 461 ± 5
    C. I. 4090_____ 469 ± 4
    Galgalos, C. I. 2398_____ 481 ± 4
    Pacific Bluestem, C. I.
      4067_____ 491 ± 4
    C. I. 4087_____ 501 ± 4
    C. I. 4087 (old seed) _____ 505 ± 3

    Average_____ 466 ± 4

Hybrid wheat:
    Jumillo × Preston_____ 417 ± 2

    Average for series_____ 431 ± 4

The water-requirement measurements for the durum wheats were 16 per cent lower than the water-requirement measurements for the common wheats. One of the durum wheats, C. I. 4131, an introduction from Siberia, showed the lowest water requirement, 357 ± 4. As in 1914, Kubanka, C. I. 1440, with a water-requirement value of 405 ± 3, ranked above all other varieties of durums except an introduction from Peru, which had a value 6 per cent higher than Kubanka. The variation of the different varieties of durum wheats based on the lowest value amounted to only 21 per cent.

Of the common wheats, the lowest water-requirement value, as in 1914, was shown by Marquis. Its value, 412 ± 3, was 15 per cent higher than the most efficient durum. The other varieties of common wheat ranged as high as 23 per cent above Marquis.

One hybrid of a durum and a common wheat was included in the test. The hybrid gave a water requirement 2 per cent lower than the mean water requirement of the two parents, 5 per cent above that of the durum parent, and 8 per cent below that of the common parent. This result does not agree with the result in 1914 when the hybrid gave a water requirement 14 per cent above the mean of the parents. Based on grain production the durum parent had a water requirement 34 per cent below that of the common wheat parent. The water requirement of the hybrid was 25 per cent above the parental mean, 4 per cent above the water requirement of the common parent, and 58 per cent above that of the durum parent. Since no explanation can be given of the result, it seems unsafe to conclude that the water requirement of wheat hybrids, based on total dry matter, would fall above that of the mean of the parents. The results obtained are more nearly in accord with those obtained with corn in 1914.

The water requirement of wheats in 1915, based on grain production, was as follows:

Durum:
    C. I. 4131 (old seed)___ 944 ± 15
    C. I. 4131_____ 1042 ± 28
    Beloturka_____ 1122 ± 11
    Beloturka (old seed)____ 1129 ± 11
    Jumillo_____ 1179 ± 15
    Kubanka, C. I. 1440___ 1232 ± 13
    C. I. 4082 (old seed)___ 1299 ± 38
    C. I. 4082_____ 1315 ± 28

    Average_____ 1158 ± 22

Common:
    Marquis (old seed)_____ 1190 ± 15
    Marquis_____ 1279 ± 22

Common—Continued.
    C. I. 4090 (old seed) __ 1451 ± 39
    Galgalos_____ 1551 ± 65
    C. I. 4090_____ 1617 ± 20
    C. I. 4087 (old seed) __ 1725 ± 25
    C. I. 4087_____ 1766 ± 39
    Preston_____ 1788 ± 71
    Pacific Bluestem_____ 3343 ± 119

    Average_____ 1746 ± 56

Hybrid:
    Jumillo × Preston____ 1859 ± 103

    Average for series___ 1491 ± 49

The water requirement of durum wheats based on grain produc-
tion was 1158 ± 22, or 66 per cent of that of the common wheats,
1746 ± 56. The order of increasing water requirement for the
varieties is almost the same as in 1914, and with a few exceptions is
approximately the same as when based on total dry matter. As
in 1914, Pacific Bluestem gave the highest water requirement among
the common wheats and C. I. 4082 among the durums. Among
the common wheats Marquis gave the lowest value during both
years. It is interesting to observe that the different varieties of
the group stand in almost the same relation to each other as they
did in 1914, and that, with one exception, the original importation
gave a slightly lower value for the water requirement, based on dry
matter, than the seed grown at the station. Except in one set,
C. I. 4090, the differences are not significant. The water require-
ment of the wheat hybrid, based on grain yield, was equal to the
mean of the parents. Detailed results of the experiments with wheat
varieties are shown in Table 8.

TABLE 8.—*Water requirement, based on dry matter and grain, of varieties of whea
at Akron, Colo., 1915*

Variety	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Kubanka, C. I. 1440 (*T. durum*).	May 22 to Aug. 21.	1	192. 4	66. 2	81. 2	34	1227	422
		2	220. 0	69. 7	92. 7	32	1330	421
		3	194. 1	61. 1	82. 0	31	1342	422
		4	222. 5	72. 2	86. 8	32	1202	390
		5	202. 1	58. 8	77. 5	29	1318	383
		6	225. 3	72. 9	87. 8	32	1204	390
Mean							1271±22	405±6
Kubanka, C. I. 1440 (*T. durum*).	May 24 to Aug. 24.	109	261. 7	93. 3	103 5	36	1109	395
		110	268. 7	91. 8	112. 0	34	1220	417
		111	246. 9	85. 8	102. 2	35	1191	414
		112	260. 2	83. 5	104. 0	32	1246	400
		113	279. 1	94. 8	111. 8	34	1179	401
		114	279. 7	93. 1	113. 4	33	1218	405
Mean							1194±13	405±3
Mean of pots 1 to 6 and 109 to 114.							1232±13	405±3
Galgalos, C. I. 2398 (*T. aesti-vum*).	May 22 to Aug. 16.	7	167. 4	56. 2	80. 4	34	1431	480
		8	159. 5	47. 7	75. 7	30	1587	475
		9	169. 1	60. 4	77. 4	36	1281	458
		10	177. 4	60. 7	87. 6	34	1443	494
		11	176. 1	55. 4	85 0	31	1534	483
		12	155. 6	38. 1	77. 4	24	2031	497
Mean							1551±65	481±4
Pacific Bluestem, C. I. 4067 (*T. aestivum*).	May 22 to Aug. 26.	13	196. 7	34. 7	95. 7	18	2758	487
		14	200. 3	30. 9	101. 6	15	3288	507
		15	190. 0	29. 9	94. 2	16	3151	496
		16	208. 1	32. 3	104. 7	16	3241	503
		17	206. 7	29. 2	97. 2	14	3329	470
		18	286. 5	32. 3	138. 5	11	4288	483
Mean							3343±119	491±4

TABLE 8.—*Water requirement, based on dry matter and grain, of varieties of wheat at Akron, Colo., 1915*—Continued

Variety	Period of growth	Pot No	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
C. I. 4087, S. P I 36499 (*T. aestivum*), old seed.	May 22 to Aug 18	19	225.9	67.1	113.9	30	1697	504
		20	208.9	60.0	104.8	29	1747	502
		21	214.5	65.7	104.0	31	1583	485
		22	219.1	65.4	110.9	30	1696	506
		23	221.3	61.8	116.7	28	1888	527
		24	225.5	65.8	114.3	29	1737	507
Mean							1725±25	505±3
C. I. 4087, S. P. I. 36499 (*T. aestivum*), Akron seed.	May 22 to Aug. 18	25	219.2	61.7	110.3	28	1788	503
		26	224.9	62.6	117.6	28	1879	523
		27	219.4	63.1	110.5	29	1751	504
		28	216.0	62.1	107.1	29	1725	496
		29	216.2	56.3	109.2	26	1940	505
		30	244.2	76.2	115.2	31	1512	472
Mean							1766±39	501±4
C. I. 4090, S. P. I 36502 (*T aestivum*), old seed.	May 22 to Aug. 16	37	219.7	74.8	97.0	34	1297	442
		38	228.8	70.8	110.3	31	1558	482
		39	214.0	66.7	98.1	31	1471	458
		40	226.1	66.0	107.8	29	1633	477
		41	231.0	74.7	107.8	32	1443	467
		42	206.1	69.4	90.5	34	1304	439
Mean							1451±39	461±5
C. I. 4090, S. P. I. 36502 (*T. aestivum*), Akron seed.	May 22 to Aug. 16	43	206.4	62.5	98.6	30	1578	478
		44	190.8	58.1	93.0	30	1601	487
		45	207.1	61.3	95.1	30	1551	459
		46	212.8	61.8	97.6	29	1579	459
		47	206.5	59.0	98.0	29	1661	475
		48	220 3	58.0	100.5	26	1733	456
Mean							1617±20	469±4
Beloturka, C. I. 3705, S. P. I. 35480 (*T. durum*), old seed.	May 22 to Aug. 21	49	313.4	105.8	119.6	34	1130	382
		50	293.4	100.0	109.5	34	1095	373
		51	273.0	96.8	107.3	35	1108	393
		52	287.1	99.5	114.6	35	1152	399
		53	313.6	108.8	119.6	35	1099	381
		54	322.3	107.1	127.4	33	1190	395
Mean							1129±11	387±3
Beloturka, C. I. 3705, S. P. I. 35480 (*T. durum*), Akron seed.	May 22 to Aug. 21	55	244.6	84.2	98.6	34	1171	403
		56	260.7	91.2	101.9	35	1117	391
		57	284.7	97.1	110 4	34	1137	388
		58	269.8	93.0	106.5	34	1145	395
		59	322.9	110.9	122.1	34	1101	378
		60	313.4	113.6	120.3	36	1059	384
Mean							1122±11	390±2
C. I. 4131, S. P. I. 37159 (*T. durum*), from Siberia, old seed.	May 28 to Aug. 24	67	336.8	132.2	119.5	39	904	355
		68	319.0	118.2	118.4	37	1002	371
		69	274.3	96.3	94.2	35	978	343
		70	312.2	123.3	111.8	39	907	358
		71	309.2	117.5	114.3	38	973	370
		72	247.4	94.4	84.9	38	899	343
Mean							944±15	357±4
C. I. 4131, S. P. I. 37159 (*T. durum*), Akron seed.	May 28 to Aug. 24	73	363.0	137.0	131.2	38	958	361
		74	355.3	124.2	138.6	35	1116	390
		75	288.1	104.5	103.8	36	993	360
		76	330.2	116.3	111.2	35	956	337
		77	322.0	112.8	124.8	35	1106	388
		78	337.6	103.7	116.4	31	1122	345
Mean							1042±28	364±6

TABLE 8.—*Water requirement, based on dry matter and grain, of varieties of wheat at Akron, Colo., 1915*—Continued

Variety	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
C. I. 4082, S. P. I. 36388 (*T. durum*), from Peru, old seed.	May 22 to Aug. 25.	85	281. 1	97. 3	104. 8	35	1077	373
		86	285. 1	96. 8	122. 6	34	1267	430
		87	275. 7	84. 9	115. 3	31	1358	418
		88	285. 0	86. 6	116. 1	30	1341	407
		89	292. 8	83. 0	124. 8	28	1504	426
		90	311. 1	104. 9	130. 9	34	1248	421
Mean							1299±38	413±6
C. I. 4082, S. P. I. 36388 (*T. durum*), Akron seed.	May 22 to Aug. 25.	91	291. 2	102. 1	128. 4	35	1258	441
		92	324. 2	109. 4	141. 3	34	1292	436
		93	322. 1	95. 3	139. 4	30	1463	433
		94	306. 6	100. 0	124. 0	33	1240	404
		95	270 2	85. 6	118. 9	32	1389	440
		96	243. 3	84. 4	105. 4	35	1249	433
Mean							1315±28	431±3
Marquis, C. I. 3641 (*T. aestivum*), old seed.	May 22 to Aug. 16.	97	198. 9	71. 3	82. 6	36	1158	415
		98	212 4	74. 4	86. 2	35	1159	406
		99	219. 8	76. 6	86. 5	35	1129	394
		100	205. 9	71. 2	86. 2	35	1211	419
		101	211. 8	69. 2	87. 2	33	1260	412
		102	199. 7	69 4	84. 7	35	1220	424
Mean							1190±15	412±3
Marquis, C. I. 3641 (*T. aestivum*), Akron seed.	May 22 to Aug. 16.	103	220. 6	71. 7	95. 4	33	1331	432
		104	200 0	64. 3	83. 9	32	1305	420
		105	199. 5	66. 1	87. 9	33	1330	441
		106	196. 2	62. 5	82. 9	32	1326	423
		107	203. 1	68 5	81. 6	34	1191	402
		108	227. 4	81. 3	96. 6	36	1188	425
Mean							1279±22	424±3
Jumillo, C. I. 1736 (*T. durum*).	May 28 to Aug. 24.	115	269. 2	91. 4	112. 9	34	1235	419
		116	271. 0	90. 7	102. 7	33	1132	379
		117	292. 9	89. 7	108. 1	31	1205	369
		118	265. 4	89. 4	109. 1	34	1220	411
		119	255. 1	88. 8	100. 3	35	1130	393
		120	269. 8	94. 7	109 2	35	1153	405
Mean							1179±15	396±6
Jumillo × Preston	May 28 to Aug. 26.	121	249. 1	69. 7	107. 2	28	1538	430
		122	243. 0	65. 5	101. 8	27	1554	419
		123	257. 4	62. 3	106. 4	24	1708	413
		124	254. 3	57. 6	104. 7	23	1818	412
		125	278. 0	50. 3	116. 0	18	2306	417
		126	303. 1	55. 9	124. 6	18	2229	411
Mean							1859±103	417±2
Preston, C. I. 3328 (*T. aestirum*).	May 28 to Aug. 26.	127	294. 2	73. 3	126. 0	25	1719	428
		128	290. 0	77. 7	127 1	27	1636	438
		129	239. 2	60. 0	112. 4	25	1873	470
		130	257. 2	58 4	119. 6	23	2048	465
		131	283. 4	66. 3	133. 2	23	2009	470
		132	308. 9	94 5	136. 4	31	1443	442
Mean							1788±71	452±6

EFFECT OF OLD SEED ON WATER REQUIREMENT

Since the results are so slight as not to affect the general average of these varieties and since they serve really only as duplicate determinations the measurements covering the effect of old seed on water requirement are included here. The new seed was pro-

duced in 1914 and the old seed was part of the original importation or was produced earlier than 1914.

A comparison of the water-requirement values of durum and common wheats from both new and old seed, on the basis of both dry matter and grain production, in 1915, shows the following results:

Variety	Water requirement based on total dry matter with—		Water requirement based on total grain with—	
	Old seed	New seed	Old seed	New seed
Durum wheats.				
C. I. 4131	357±4	364±6	944±15	1042±28
Beloturka, C. I. 3705	387±3	390±2	1122±11	1129±11
C. I. 4082	413±6	431±3	1315±28	1299±38
Common wheats:				
Marquis, C. I. 3641	412±3	424±3	1190±15	1279±22
C. I. 4090	461±5	469±4	1451±39	1617±20
C. I. 4087	505±3	501±4	1725±25	1766±39
Mean	423±4	430±4	1291±24	1355±28

The differences are very slight but except in one case are all in one direction, the old seed giving the lower water requirement.

A similar experiment was conducted in 1916 except that old seed was used, and was compared with "first generation" seed produced in 1914, and with "second generation" seed produced in 1915. The water-requirement measurements were as follows:

Variety	Water requirement based on total dry matter with—			Water requirement based on grain with—		
	Old seed	First generation seed	Second generation seed	Old seed	First generation seed	Second generation seed
Durum wheats.						
C. I. 4082	666±24	710±9	683±18	2291±172	1903±80	1821±60
C. I. 4131	719±15	717±30	712±10	2126±104	2640±170	2095±104
Common wheats:						
Marquis, C. I. 3641	680±12	726±5	713±19	2395±230	2339±126	2008±43
Mean	688±18	718±18	703±16	2271±176	2294±131	1975±74

WATER REQUIREMENT OF BARLEY, OATS, AND RYE, 1915

Barley gave a water requirement of 404 ± 11 and rye of 469 ± 8. Barley has a water requirement, when based on grain production, of 949 ± 50, equal to the best of the wheats.

Two varieties of oats were grown in 1915, Burt with a water requirement of 445 ± 5, and Swedish Select with a water requirement of 448 ± 10, a difference of less than 1 per cent (Table 9). As compared with wheat, oats had a water requirement 4 per cent higher. On the basis of grain produced, the water requirement of Burt oats was 1150 ± 27 and of Swedish Select 1102 ± 34, both somewhat lower than in 1914 but about the same as the durum wheats.

TABLE 9.—*Water requirement, based on grain and dry matter, of barley, oats, and rye at Akron, Colo., 1915*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Barley, Hannchen, C. I. 531 (*Hordeum distichon*).	May 28 to Aug. 11	133	204.8	88.5	72.8	43	823	355
		134	283.7	131.5	107.4	46	817	379
		135	271.9	131 0	105.8	48	808	389
		136	274.1	106.6	121.9	39	1144	445
		137	285.2	116.0	114.9	41	991	403
		138	250.4	102.0	113.4	41	1112	453
Mean							949±50	404±11
Oats, Swedish Select, C. I. 134 (*Avena satina*).	May 28 to Aug. 21	139	317.5	128 8	136.9	41	1063	431
		140	349.1	149.0	144 6	43	970	414
		141	307.2	128.1	147.4	42	1151	480
		142	284.0	107.3	139.3	38	1298	490
		143	363.2	144.8	163.2	40	1127	449
		144	373.5	158.4	158.5	42	1001	424
Mean							1102±34	448±10
Oats, Burt, C. I. 293 (*A. satiia*).	May 28 to Aug. 11	145	274.5	98.8	122.7	36	1242	447
		146	222.3	82.9	99.5	37	1200	448
		147	203.4	80.1	97.7	39	1220	480
		148	213.2	88.9	95.0	42	1069	446
		149	209.3	82.8	90.1	40	1088	430
		150	273.8	106.7	115.3	39	1081	421
Mean							1150±27	445±5.
Rye, Vern, C. I. 73 (*Secale cereale*).	May 28 to Aug. 17	151	222.0	71.8	101 9	32	1419	459
		152	205.8	71.6	92.8	35	1296	451
		153	178.7	54.2	88.7	30	1637	496
		154	207.8	80.9	89.5	39	1106	431
		155	206.1	62.7	98.5	30	1571	478
		156	196.8	51.9	97.6	26	1881	496
Mean							1485±80	469±8

As in previous experiments rye was the least efficient in the use of water of any of the small grains. It was followed by the common wheats, oats, barley, and durum wheats in the order of their increased efficiency. On the basis of grain production barley is the lowest, followed by durum wheat, oats, rye, and common wheats. It is possible that the relatively high water requirement of barley and the durum wheats is due to the cool, damp season, which was less favorable for their growth than for some of the other varieties.

WATER REQUIREMENT OF FLAX, 1915

Six varieties of flax were grown in 1915. (See fig. 4 and Table 10.) The water requirement of each variety, based on the production of dry matter, is as follows:

Kashgar	569±4	Smyrna	663±16
North Dakota Resistant	579±10	Jalaun	682±14
Reserve	615±7		
Soddo White	625±4	Average	622±10

Flax has a high water requirement, about equivalent to alfalfa, and higher than any of the small grains. The range in water requirement of the varieties here tested, based on the lowest value, is 20 per cent.

TABLE 10.—*Water requirement, based on grain and dry matter, of flax varieties, at Akron, Colo., 1915*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
FLAX								
(*Linum usitatissimum*)			*Grams*	*Grams*	*Kgms.*	*P. ct.*		
North Dakota Resistant, No. 114, C. I. 13.	June 1 to Aug. 17__	157	140.0	40.4	75.5	29	1869	539
		158	150.5	30.4	82.2	20	2704	546
		159	142.3	43.7	91.4	31	2092	642
		160	166.5	55.2	97.8	33	1772	587
		161	141.3	40.0	81.1	28	2028	574
		162	152.5	48.1	89.4	32	1859	586
Mean_____							2054±87	579±10
Smyrna, S. P. I. 36949 (Turkey), C. I 30.	June 1 to Aug. 26__	163	169.1	42.1	114.3	25	2715	676
		164	195.9	52.3	147.9	27	2828	755
		165	166.8	36.7	103.0	22	2807	618
		166	175.0	32.4	109.1	19	3367	623
		167	178.3	45.9	111.1	26	2420	623
		168	203.7	53.9	138.7	26	2573	681
Mean_____							2785±82	663±16
Soddo White, S. P. I. 37086 (Abyssinia), C. I. 36.	June 1 to Aug. 17__	169	169.1	66.4	107.2	39	1614	634
		170	139.4	56.6	87.5	41	1546	628
		171	167.8	72.2	107.3	43	1486	639
		172	162.2	64.5	100.8	40	1563	621
		173	169.8	67.3	107.5	40	1597	633
		174	163.0	65.3	96.6	40	1479	593
Mean_____							1548±17	625±4
Jalaun, S. P. I. 36566 (India), C. I. 21.	June 1 to Aug. 17__	175	119.4	38.2	83.8	32	2194	702
		176	104.1	27.4	70.2	26	2562	674
		177	89.5	24.2	62.6	27	2587	699
		178	116.6	39.7	81.4	34	2050	698
		179	34.4	.8	19.9			578
		180	94.6	25.8	69.8	27	2705	735
Mean_____							2420±101	682±14
Kashgar, S. P. I. 37719, C. I. 50-1.	June 1 to Sept. 2__	181	168.3	25.2	99.2	15	3937	589
		182	175.8	25.6	102.7	15	4012	584
		183	170.0	10.6	96.1	6	ª9066	565
		184	141.7	1.7	80.7			570
		185	179.4	14.3	99.3	8	ª6944	554
		186	185.7	16.2	102.9	9	ª6352	554
Mean_____							3975±32	569±4
Reserve, C. I. 19_____	June 1 to Aug. 26__	187	172.1	45.0	99 9	26	2220	•580
		188	180.3	49.6	113 9	28	2296	632
		189	189.1	45.3	121.1	24	2673	640
		190	177.2	46 0	110.3	26	2398	622
		191	195.4	53.2	116.7	27	2194	598
		192	200.6	48 8	124.2	24	2545	619
Mean_____							2388±57	615±7

ª Omitted in computing mean.

Based on grain production the water requirement of flax was as follows:

Soddo White	1548±17	Smyrna	2785±82
North Dakota Resistant	2054±87	Kashgar	3975±32
Reserve	2388±57		
Jalaun	2420±101	Average	2528±70

The range is very great, the most efficient requiring less than two-fifths as much water as the least efficient, and ranking in grain production with some of the better common wheats.

Fig. 4.—Flax grown in the water requirement experiments at Akron, Colo., in 1915. Photographed July 13, 1915

WATER REQUIREMENT OF CORN, SORGHUM, SUDAN GRASS, AND MILLET, 1915

The values obtained for these crops in 1915 were as follows:

Millet	202±1	Corn, Northwestern Dent	253±7
Sorghum, Minnesota Amber	203±3	Sudan grass	260±3

Millet and sorghum gave about the same result while corn required 25 per cent, and Sudan grass 28 per cent more water than millet and sorghum. Millet and sorghum have the lowest water requirement of any of the crops grown. The water requirement based on grain produced was lowest in millet, 665±24, highest in corn, 2060±108, and intermediate for sorghum, 1116±105. The value for millet was the lowest recorded, while the water requirement of sorghum was about as low as for the durum wheats. Table 11 summarizes the results of this experiment.

TABLE 11.—*Water requirement, based on grain and dry matter, of millet, sorghum, sudan grass, and corn, at Akron, Colo., 1915*

| Kind of plant | Period of growth | Pot No. | Dry matter | Grain | Water | Grain | Water requirements based on— | |
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Millet, Kursk, S. P. I. 34771 (*Chaetochloa italica*).	June 26 to Sept. 2	223	248.1	89.1	49.4	36	554	199
		224	271.1	92.8	54.4	34	586	201
		225	271.0	78.3	54.8	29	700	202
		226	304.1	73.4	59.5	24	811	196
		227	241.5	73.3	48.9	30	667	202
		228	200.0	63.2	42.4	32	671	212
Mean							665±24	202±1
Sorghum, Minnesota Amber, A. D. I. 341-13 (*Andropogon sorghum*).	June 26 to Sept. 14	235	182.4	47.5	36.0	26	758	197
		236	202.0	33.1	44.5	16	1344	220
		237	244.5	56.0	49.8	23	889	204
		238	153.9	20.6	32.0	13	1553	208
		239	177.3	40.5	35.2	23	869	199
		240	258.8	38.7	49.7	15	1284	192
Mean							1116±105	203±3
Sudan grass (*Andropogon sorghum aethiopicus*): S. P. I. 25017 (in the shelter) (first crop).	June 26 to Aug 28	253	156.2		39.8			255
		254	150.8		36.4			241
		255	156.9		39.1			249
		256	163.1		41.9			257
		257	203.6		48.8			240
		258	222.6		52.4			235
Mean								246±3
S. P. I. 25017 (second crop).	Aug. 29 to Sept. 24	253	16.3		6.8			417
		254	17.9		6.9			385
		255	15.8		6.2			392
		256	12.5		6.0			480
		257	16.6		6.7			404
		258	10.3		4.8			466
Mean								424±12
S. P. I. 25017 (combined crop).	June 26 to Sept. 24	253	172.5		46.6			270
		254	168.7		43.3			257
		255	172.7		45.3			262
		256	175.6		47.9			273
		257	220.2		55.5			252
		258	232.9		57.2			246
Mean								260±3
Corn, Northwestern Dent (*Zea mays*).	June 26 to Sept. 14	241	71.9	8.5	18.6	12	2188	259
		242	76.8		16.1			210
		243	67.0		18.2			272
		244	159.5	20.5	39.6	13	1932	248
		245	173.7	7.0	43.3	4		249
		246	123.2		34.8			282
Mean							2060±108	253±7

WATER REQUIREMENT OF LEGUMES, 1915

Of two closely related varieties of alfalfa grown, one gave a value of 695 ± 9 and the other a value about 1 per cent less, 685 ± 13 (Table 12). Cowpeas required only 413 ± 5 or 60 per cent of that of alfalfa. The water requirement of cowpeas, based on seed production, was 1257 ± 39, almost as low as the durum wheats. A second set of cowpeas planted later required 17 per cent more water than the regular crop and produced little seed.

TABLE 12.—*Water requirement, based on dry matter, of legumes at Akron, Colo., 1915*

Kind of plant	Period of growth	Pot No	Dry matter	Grain	Water	Grain	Water requirements based on— Grain	Dry matter
ALFALFA								
(*Medicago sativa*)			*Grams*	*Grams*	*Kgms*	*P ct.*		
A. D. I. E-23, first crop_____	June 17 to Aug. 6__	193	163.7	_____	113.0	_____	_____	690
		194	126.8	_____	79.2	_____	_____	625
		195	133.7	_____	95.2	_____	_____	712
		196	119.7	_____	73.3	_____	_____	612
		197	117.2	_____	74.7	_____	_____	637
		198	138.0	_____	83.0	_____	_____	601
Mean_____		____	_____	_____	_____	_____	_____	646±14
A. D. I. E-23, second crop_	Aug. 7 to Sept. 21__	193	88.9	_____	69.6	_____	_____	783
		194	98.1	_____	74.9	_____	_____	764
		195	80.9	_____	64.3	_____	_____	795
		196	74.3	_____	54.8	_____	_____	738
		197	79.6	_____	61.9	_____	_____	778
		198	78.3	_____	61.2	_____	_____	782
Mean_____		____	_____	_____	_____	_____	_____	773±6
A. D. I. E-23 combined crop.	June 17 to Sept. 21__	193	252.6	_____	182.6	_____	_____	723
		194	224.9	_____	154.1	_____	_____	685
		195	214.6	_____	159.5	_____	_____	743
		196	194.0	_____	128.1	_____	_____	660
		197	196.8	_____	136.6	_____	_____	694
		198	216.3	_____	144.2	_____	_____	667
Mean_____		____	_____	_____	_____	_____	_____	695±9
A. D. I. 162-98-Al, first crop.	June 17 to Aug 6__	199	93.3	_____	53.8	_____	_____	577
		200	138.5	_____	78.5	_____	_____	567
		201	133.3	_____	82.8	_____	_____	621
		202	120.1	_____	74.0	_____	_____	616
		203	77.9	_____	55.8	_____	_____	716
		204	124.6	_____	76.8	_____	_____	616
Mean_____		____	_____	_____	_____	_____	_____	619±13
A. D. I. 162-98-Al, second crop.	Aug. 7 to Sept. 21__	199	66.2	_____	53.1	_____	_____	802
		200	86.7	_____	61.9	_____	_____	714
		201	82.7	_____	64.3	_____	_____	778
		202	73.1	_____	58.6	_____	_____	802
		203	69.6	_____	60.5	_____	_____	869
		204	88.2	_____	63.9	_____	_____	724
Mean_____		____	_____	_____	_____	_____	_____	782±16
A. D. I. 162-98-Al, combined crop.	June 17 to Sept. 21__	199	159.5	_____	106.9	_____	_____	670
		200	225.2	_____	140.4	_____	_____	623
		201	216.0	_____	147.1	_____	_____	681
		202	193.2	_____	132.6	_____	_____	686
		203	147.5	_____	116.3	_____	_____	788
		204	212.8	_____	140.7	_____	_____	661
Mean_____		____	_____	_____	_____	_____	_____	685±13
COWPEA								
(*Vigna sinensis*)								
S. P. I. 29282_____	June 4 to Sept. 9__	217	126.3	39.2	51.1	31	1304	405
		218	119.8	40.8	51.0	34	1250	426
		219	105.9	41.2	41.5	39	1007	392
		220	152.5	42.8	64.7	28	1512	424
		221	128.3	43.0	51.7	34	1202	403
		222	133.8	44.9	57.0	34	1269	426
Mean_____		____	_____	_____	_____	_____	1257±39	413±5
S. P. I. 29282_____	July 21 to Sept. 21__	259	73.4	3.4	39.2	5	ₐ11529	534
		260	79.8	2.9	39.0	4	ₐ13448	489
		261	96.6	9.1	45.2	9	4967	468
		262	65.8	5.0	32.2	8	6440	489
		263	101.6	7.3	46.8	7	6411	461
		264	76.9	2.6	35.1	3	ₐ13500	456 ₐ
Mean_____		____	_____	_____	_____	_____	5939±3882	483±8

ₐOmitted in calculating the mean.

WATER REQUIREMENT OF POTATOES, COTTON, GRAMA GRASS, AND PIGWEED, 1915

Potatoes had a water requirement of 329±4, between oats and wheat (Table 13). On the basis of tuber production the value was 945±134, and on the basis of green weight it would be about 192±29. During 1915 cotton had a very high water requirement, 443±8, or 35 per cent above that of potatoes, due largely to the cool, damp year, which was almost ideal for potato, but very unfavorable for cotton.

TABLE 13.—*Water requirement, based on tuber and dry matter, of potatoes, and on dry matter in cotton, grama grass, and pigweed, at Akron, Colo., 1915*

Kind of plant	Period of growth	Pot No.	Dry matter	Tubers	Water	Tubers	Water requirements based on—	
							Grain	Dry matter
POTATO				*Grams*	*Grams*	*Kgms.*	*Per cent*	
(*Solanum tuberosum*)								
Irish Cobbler	July to Sept. 25	307	56.3	25.1	19.2	45	765	341
		308	60.6	27.1	20.5	45	756	338
		309	67.2	16.1	22.3	24	1385	332
		310	66.9	10.6	22.8	16	*a* 2151	341
		311	85.6	48.8	25.5	57	523	298
		312	63.4	15.9	20.6	25	1296	325
Mean							945±134	329±4
COTTON								
(*Gossypium hirsutum*)								
Triumph	June 26 to Sept. 25	229	82.0		38.7			472
		230	74.4		35.2			473
		231	122.0		51.9			425
		232	141.5		63.3			447
		233	127.5		52.2			409
		234	104.6		45.0			430
Mean								443±8
GRAMA GRASS								
(*Bouteloua gracilis*)								
First crop	June 4 to Aug 6	205	23.2		6.9			297
		206	18.2		5.2			286
		207	28.9		8.0			277
		208	38.1		10.2			268
		209	44.7		12.4			277
		210	22.4		6.0			268
Mean								279±3
Second crop	Aug. 6 to Sept. 21	205	11.3		5.9			522
		206	8.8		4.1			466
		207	11.1		5.3			477
		208	23.9		5.7			238
		209	26.0		7.2			277
		210	13.3		4.4			331
Mean								385±39
Combined crop	June 4 to Sept. 21	205	34.5		12.8			371
		206	27.0		9.3			344
		207	40.0		13.3			333
		208	62.0		15.9			256
		209	70.7		19.6			277
		210	35.7		10.4			291
Mean								312±14

a Not included in the mean.

Table 13.—*Water requirement, based on tuber and dry matter, of potatoes, and on dry matter in cotton, grama grass, and pigweed, at Akron, Colo., 1915*—Contd.

| Kind of plant | Period of growth | Pot No | Dry matter | Tu-bers | Water | Tu-bers | Water requirements based on— | |
							Grain	Dry matter
PIGWEED								
(Amaranthus retroflexus)			*Grams*	*Grams*	*Kgms*	*P. ct.*		
First crop	June 26 to Aug. 6	211	116. 2		28. 1			242
		212	128. 0		32. 0			250
		213	160. 3		36. 2			226
		214	112. 8		28 3			251
		215	113. 1		27. 1			240
		216	145. 5		32. 6			224
Mean								239±3
Second crop	Aug. 6 to Sept. 21	211	29. 0		5 5			190
		212	28 7		5. 9			206
		213	19. 7		4. 0			203
		214	37. 1		6. 8			183
		215	39 6		7. 2			182
		216	24. 3		4. 0			165
Mean								188±4
Combined crop	June 26 to Sept. 21	211	145. 2		33. 6			231
		212	156. 7		37. 9			242
		213	180. 0		40. 2			223
		214	149 9		35. 1			234
		215	152. 7		34. 3			225
		216	169 8		36. 6			216
Mean								229±3

Pigweed produced only two crops with a water requirement of 229 ± 3, while grama grass also produced two crops and had a water requirement of 312 ± 14. During 1914 pigweed was 21 per cent and during 1915 27 per cent more efficient than grama grass. As compared with sorghum and millet, pigweed required 13 per cent and grama grass 54 per cent more water.

SUMMARY OF WATER REQUIREMENT MEASUREMENTS MADE IN 1915

The climatic conditions in 1915 were unusually cool and damp as compared with average years. (See Tables 27, 28, 31, and 32.) The evaporation, 33.40 inches for the growing period, was lower than for any year from 1908 to 1924, and the precipitation was unusually heavy, 19.44 inches for the six months' growing period. The water-requirement value in 1915 (see Table 28) was 27 points lower than 1914, 45 points lower than 1916, and 20 points lower than 1917. A comparison of the water requirements of the 1915 crops with those of 1914, shows that cowpeas was the outstanding crop of 1915, with a water requirement 43 points lower than in 1914, while the 15 crops grown for the period, 1914 to 1917, ranged from 19 to 43 points lower in their water requirement in 1915 than in 1914.

Of the crops grown during the period 1914 to 1917, the lowest proportionate values in 1915 were for corn, 8 points below the mean of all crops, followed by Kursk millet, Swedish Select and Burt oats, cowpeas, and Sudan grass. The highest relative value was for grama grass, 17 points above the mean for all crops, followed by Galgalos wheat, cotton, alfalfa, and Kubanka wheat.

On the basis of grain production the water requirement was lower in 1915 than in any other year except 1912. (See Table 29.) In general, the plants which have a low water requirement on the basis of dry matter, also have a low water requirement when based on grain yield. Oats was proportionately low and sorghum proportionately high in 1915.

WATER REQUIREMENT OF PLANTS, 1916

Of the plants grown at Akron in 1916 35 sets are included in these tables. The year was unusually dry, the precipitation for the growing period being 10.77 inches, the lowest for any year of the period, 1911 to 1917, with the exception of 1911. Evaporation, 47.18 inches for the growing period, was correspondingly high. Although the evaporation for the season was as high in 1911 as in 1916, the monthly evaporation was more evenly distributed in 1911. In July, 1916, the evaporation was higher than in any month during the period 1908 to 1924, inclusive. (See Tables 27, 28, 31, and 32.) This unusually dry month is reflected in the high water requirement, since practically all crops were actively growing during this period.

WATER REQUIREMENT OF VARIETIES OF WHEAT, 1916

Fifteen sets of wheat were grown in 1916, including 8 durum, 6 of common, and 1 of a hybrid wheat. The water-requirement results, based on dry matter, are as follows:

Durum:
    Kubanka, C. I. 1440____ 636 ± 14
    C. I. 4082 (old seed)____ 666 ± 24
    C. I. 4082 (second genera-
      tion seed)_____ 683 ± 18
    Kubanka, C. I. 2094____ 696 ± 30
    C. I. 4082 (first genera-
      tion seed)_____ 710 ± 9
    C. I. 4131 (second gen-
      eration seed_____ 712 ± 10
    C. I. 4131 (first genera-
      tion seed)_____ 717 ± 30
    C. I. 4131 (old seed)_____ 719 ± 15

    Average_____ 692 ± 20

Common:
    Galgalos, C. I. 2398_____ 652 ± 8
    Marquis, C. I. 3641 (old
      seed)_____ 680 ± 12

Common—Continued.
    Pacific Bluestem, C. I.
      4067_____ 690 ± 7
    Marquis, C. I. 3641 (sec-
      ond generation seed) __ 713 ± 19
    Marquis, C. I. 3641 (first
      generation seed)_____ 726 ± 5
    Haynes Bluestem_____ 743 ± 11

    Average_____ 701 ± 11

Hybrid:
    Kubanka, C. I. 2094 ×
      Haynes Bluestem_____ 636 ± 21

    Average for series_____ 692 ± 17

The water requirement for the durum wheats was 1 per cent lower than the water requirement for the common wheats. In contrast to 1914 and 1915, Kubanka had the lowest water requirement of any of the varieties of wheat grown. The variation between the lowest and highest water-requirement measurements of the durums was 13 per cent. The lowest value among the common wheats was obtained from Galgalos, 652 ± 8, which was 2 per cent higher than the most efficient durum. The varieties of common wheat showed a range of 14 per cent in water-requirement measurements.

The hybrid wheat had a water requirement 14 per cent lower than that of the common parent, 9 per cent lower than that of the durum parent, and 12 per cent lower than the mean of the parents. These results show a marked improvement on the part of the hybrid, an improvement equal to that shown by corn hybrids. Among the

corn hybrids the water-requirement range was from 10 per cent above to 10 per cent below the parental mean. The water-requirement measurements for the wheat 'hybrids recorded in this paper range from 14 per cent below to 14 per cent above the parental mean. On the basis of grain production the hybrid requirement is 16 per cent more than the parental mean.

The water requirement measurements of the wheats, based on grain production, are as follows:

Durum wheats
    Kubanka, C. I. 1440__ 1779 ± 108
    C. I. 4082 (second gen-
       eration seed)_____ 1821 ± 60
    Kubanka, C. I. 2094__ 1857 ± 142
    C. I. 4082 (first genera-
       tion seed)_____ 1903 ± 80
    C. I. 4131 (second gen-
       eration seed)_____ 2095 ± 104
    C. I. 4131 (old seed)__ 2125 ± 104
    C. I. 4082 (old seed)__ 2291 ± 172
    C. I. 4131 (first genera-
       tion seed)_____ 2640 ± 170
      Average_____ 2064 ± 123

Common wheats:
    Marquis (second gen-
       eration seed)_____ 2008 ± 43

Common wheats—Contd.
    Haynes Bluestem_____ 2338 ± 78
    Marquis (first genera-
       tion seed)_____ 2339 ± 126
    Marquis (old seed)____ 2395 ± 232
    Galgalos, C. I. 2398___ 2445 ± 155
    Pacific Bluestem, C. I.
      4067_____ 2959 ± 346

      Average_____ 2414 ± 192

Hybrid:
    Kubanka, C. I. 2094 ✕
      Haynes Bluestem___ 2434 ± 103

      Average for series__ 2229 ± 152

The water requirement of the durum wheats in 1916 was 2064 ± 123, or 85 per cent of the common wheats. Kubanka, C. I. 1440, gave the lowest water requirement of any of the varieties grown, while Pacific Bluestem had the highest value. One of the sets of Marquis wheat again gave the lowest water requirement of the common wheats. The range in water requirement was about the same in both groups, the lowest value being about 68 per cent of the highest. On the basis of total dry matter the water-requirement range in the durum group was also the same as in the common group. Based on grain production, however, the water requirement of the durums was much less, the lowest value being 88 per cent of the highest. Table 14 gives the water-requirement results for the wheat varieties in 1916.

TABLE 14.—*Water requirement, based on grain and dry matter, of wheat varieties at Akron, Colo., 1916*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Kubanka, C. I. 1440 (*Triticum durum*).	June 3 to Aug. 12_	1	89. 6	29. 2	62. 3	33	2134	695
		2	104. 3	38. 7	64. 0	37	1654	614
		3	85. 8	28. 9	48. 8	34	1689	569
		4	95. 7	35. 8	62. 5	37	1746	653
		5	102. 1	39. 7	68. 2	39	1718	668
		6	72. 8	26. 0	45. 0	36	1731	618
Mean_____							1779±108	636±14
Galgalos, C. I. 2398 (*T. durum*).	June 3 to Aug. 12_	7	59. 9	17. 2	40. 6	29	2360	678
		8	44. 5	9. 5	27. 9	21	2937	627
		9	45. 6	9. 4	28. 0	21	2979	614
		10	27. 8	6. 9	18. 3	25	2652	658
		11	90 3	30. 0	58. 9	33	1963	652
		12	70. 2	27. 0	48. 1	38	1781	685
Mean_____							2445±155	652±8

TABLE 14.—*Water requirement, based on grain and dry matter, of wheat varieties at Akron, Colo., 1916*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P ct.*		
Pacific Bluestem, C. I. 4067 (*T. aestivum*).	June 3 to Aug. 21	13	131 0	40.7	91.3	31	2243	697
		14	47.1	4.4	30.8	----	----	654
		15	116 4	28.5	80 0	24	2807	687
		16	90.0	16.9	64.7	19	3828	719
		18	54.5	1.5	37.7	----	----	692
Mean							2959±346	690±7
C. I. 4131 (from Siberia) (*T. durum*), old seed.	June 3 to Aug. 16	19	97.5	36.4	65 3	37	1794	670
		20	86.5	25.9	59.5	30	2297	688
		21	39.8	11.1	27.6	28	2486	693
		22	76.2	30 4	56.0	40	1842	735
		23	70.8	22.8	50.3	32	2206	710
		24	27.2	6 5	22 3	24	*a*3431	820
Mean							2125±104	719±15
C. I. 4131 (from Siberia), first generation seed.	June 3 to Aug. 16	25	14.1	3 7	12 6	26	*a*3405	894
		26	79 3	22 6	49.7	32	2199	707
		27	33.9	7 3	23.9	22	3274	705
		28	30 2	7 8	18 6	26	2385	616
		29	39.4	9 7	26.2	25	2701	665
Mean							2640±170	717±30
C. I. 4131 (from Siberia), second generation seed.	June 3 to Aug. 16	32	32 5	9.4	23.0	29	2447	708
		33	78.8	30.4	58.9	39	1938	747
		34	89 7	36.6	66.0	41	1803	736
		35	77.3	26 7	51.5	35	1929	666
		36	44.1	13.2	31.1	20	2356	705
Mean							2095±104	712±10
C. I. 4082 (from Peru) (*T durum*), old seed.	June 3 to Aug. 21	43	36.4	11.5	23.5	32	2043	646
		44	22.7	6.7	17.3	30	2582	762
		45	18.2	3.9	11.4	21	2923	626
		46	25.9	10.0	15.2	39	1520	587
		47	49.4	14.7	35.1	30	2288	711
Mean							2291±172	666±24
C. I. 4082 (from Peru), first generation seed.	June 3 to Aug. 21	49	53.8	21.5	39.1	40	1819	727
		50	27.1	2.8	19 3	10	*a*6893	712
		51	96.2	40 3	67 1	42	1665	698
		52	37 5	11.4	26.1	30	2289	696
		53	62.3	20.8	41.4	33	1990	665
		54	100.0	43.5	76.3	44	1754	763
Mean							1903±80	710±9
C. I. 4082 (from Peru), second generation seed.	June 3 to Aug. 21	55	24 5	7.2	15 9	29	2208	649
		56	88.7	37.4	58.9	42	1575	664
		57	26.7	11.4	20.6	43	1807	772
		58	26.7	9.5	16.9	36	1779	633
		59	50.9	17.2	32.8	34	1907	644
		60	101.5	45.2	74.5	45	1648	734
Mean							1821±68	683±18
Marquis, C. I. 3641 (*T. durum*), old seed.	June 3 to Aug. 9	67	38.4	8.2	24.4	21	2976	635
		68	86 8	30.2	60 1	35	1990	692
		70	77 9	25 1	55 7	32	2219	715
		72	49.1	9 8	33.2	20	*a*3388	676
Mean							2395±232	680±12

a Omitted in the mean

TABLE 14.—*Water requirement, based on grain and dry matter, of wheat varieties at Akron, Colo., 1916*—Continued

Kind of plant	Period of growth	Pot No	Dry matter	Grain	Water	Gram	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Marquis, C. I. 3641, first generation seed.	June 3 to Aug 9	73	78.5	26.5	56.3	34	2125	717
		74	92.3	36.0	66.3	39	1842	718
		75	56.5	17.7	39.8	31	2249	704
		76	86.1	29.0	62.7	34	2162	728
		77	35.2	11.2	25.9	32	2313	736
		78	32.4	7.3	24.4	23	3342	753
Mean							2339±126	726±5
Marquis, C. I. 3641, second generation seed	June 3 to Aug. 9	79	19.5	4.0	15.7	21	a3925	805
		80	22.3	8.2	17.2	37	2098	771
		81	78.5	27.1	54.9	35	2026	699
		82	54.7	17.1	36.8	31	2152	673
		83	70.1	24.2	45.9	35	1897	655
		84	77.6	28.0	52.3	36	1868	674
Mean							2008±43	713±19
Kubanka, C. I. 2094 (*T. durum*).	June 3 to Aug. 16	91	32.3	9.5	23.4	29	a2463	724
		92	54.5	15.8	31.3	29	1981	574
		94	13.5	5.0	10.2	37	2040	756
		96	8.5	4.0	6.2	47	1550	729
Mean							1857±142	696±30
Kubanka, C. I. 2094×Haynes Bluestem, C. I. 2874.	June 3 to Aug. 16	97	65.4	18.0	39.0	28	2167	596
		98	94.8	29.5	61.7	31	2092	651
		99	92.7	25.4	56.5	27	2224	609
		100	77.8	19.5	48.7	25	2497	626
		101	36.7	7.1	20.1	19	2831	548
		102	78.9	22.2	62.0	28	2793	786
Mean							2434±103	636±21
Haynes Bluestem, Minnesota 169, C. I. 2874 (*T. aestivum*).	June 3 to Aug. 9	115	97.5	32.3	74.1	33	2294	760
		116	96.6	33.1	68.8	34	2079	712
		117	96.5	32.0	71.1	33	2222	737
		118	99.1	32.0	68.5	32	2141	691
		119	95.8	30.4	76.3	32	2510	796
		120	68.9	18.9	52.6	27	2783	763
Mean							2338±78	713±11

a Omitted in the mean.

WATER REQUIREMENT OF BARLEY, OATS, AND RYE, 1916

Barley gave a water requirement of 664 ± 9 and rye of 800 ± 11, or 64 and 71 per cent higher, respectively, than in 1915. Based on grain weight, the water requirement of barley was 1425 ± 27 and of rye 2871 ± 99, or 50 and 93 per cent higher, respectively, than in 1915.

Two varieties of oats were grown in 1916, Burt, with a water requirement of 809 ± 5, and Swedish Select, with a water requirement of 876 ± 21. Oats had a water requirement in 1916, 88 per cent higher than in 1915. As compared with wheat, oats had a water requirement 22 per cent higher. On the basis of grain production, Burt oats had a water requirement of 1975 ± 49, or 72 per cent higher than in 1915, while Swedish Select required 2288 ± 39, more than twice as much as in 1915 (Table 15).

TABLE 15.—*Water requirement, based on grain and dry matter, of barley, oats, and rye at Akron, Colo., 1916*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Barley, Hannchen, C. I. 531 (*Hordeum distichon*).	June 3 to July 29	139	82.2	40.7	58.1	50	1428	707
		140	103.2	45.2	71.0	44	1571	688
		141	113.5	55.2	74.3	49	1346	655
		142	108.5	52.0	68.2	48	1312	629
		143	112.2	52.5	73.8	47	1406	658
		144	102.5	44.5	66.2	43	1488	646
Mean							1425±27	664±9
Oats, Swedish Select, C. I. 134 (*Avena sativa*).	June 3 to Aug. 9	127	104.0	42.2	95.5	40	2263	918
		128	97.7	40.2	93.6	41	2328	958
		129	108.1	38.8	98.4	36	2536	910
		130	98.3	34.6	79.0	35	2283	804
		131	106.7	40.7	93.9	38	2307	880
		132	104.6	40.7	81.9	39	2012	783
Mean							2288±39	876±21
Oats, Burt, C. I. 293 (*A. sativa*).	June 3 to July 26	133	90.4	39.8	71.9	44	1807	795
		134	86.0	38.3	67.4	45	1760	784
		135	80.0	33.0	64.9	41	1967	811
		136	85.4	35.2	70.7	41	2009	828
		137	82.3	33.3	68.1	40	2045	827
		138	88.7	31.6	71.5	36	2263	806
Mean							1975±49	809±5
Rye, Vern, C. I. 73 (*Secale cereale*).	June 3 to Aug. 5	145	84.5	22.3	70.4	26	3157	833
		146	91.7	27.2	78.1	30	2871	852
		147	89.1	20.8	69.9	23	3361	785
		148	94.4	34.1	71.6	36	2100	758
		149	56.9	16.0	45.7	28	2856	803
		150	85.5	22.9	65.9	27	2878	771
Mean							2871±99	800±11

Swedish Select oats had the highest water requirement based on total dry matter of any of the small grains, and was followed by Burt oats, rye, the common and durum wheats, and barley. Kubanka and Galgalos wheat and barley had the lowest water requirements of any of the small grains. Oats and rye seem to have been affected more than any other crop by the extremely dry season.

WATER REQUIREMENT OF CORN, SORGHUM, SUDAN GRASS, AND MILLET, 1916

Only one variety of each of these crops was grown in 1916 at Akron. The water-requirement results obtained from this test were as follows:

Sorghum, Minnesota Amber__ 296±4 | Sudan Grass_____ 426±3
Millett, Kursk_____ 367±4 | Corn, Northwestern Dent___ 495±13

This year of high water requirement did not greatly affect sorghum. (See Table 16.) Although 46 per cent higher than in 1915, its water requirement was relatively low. A similiar response has been noted for sorghum on several occasions, which during hot weather compensates in growth for the increased rate of transpiration. Sudan grass behaved much as did sorghum but showed a slightly greater reaction to the dry season, the water requirement being 64 per cent higher than in 1915. Millet, with a water requirement 82 per cent and corn and Swedish Select oats with a water requirement 96 per cent above that in 1915, responded to the dry season to a greater extent than any other crops.

TABLE 16.—*Water requirement, based on grain, of corn, sorghum, and millet, and on dry matter for sudan grass, at Akron, Colo., 1916*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct*		
Corn, Northwestern Dent (*Zea mays*).	June 16 to Aug. 23.	271	152.9	19.6	70.8	13	3612	463
		272	193.4	Trace.	100.0			517
		273	206.7	Trace.	99.1			479
		274	148.4	18 0	65.8	12	3656	443
		275	186 0	8.9	97.5	5	a10955	524
		276	196.0	2.5	106.8		a4272	545
Mean							3634±19	495±13
Sorghum, Minnesota Amber, A. D. I. 341-13 (*Andropogon sorghum*).	June 17 to Aug 23.	259	228 6	77.5	70.6	34	911	309
		260	202.1	73.6	61.4	36	834	304
		261	268.5	92.0	80.1	34	871	298
		262	238.5	94.5	67.8	40	717	284
		263	218.8	67.4	62.7	31	930	287
Mean							853±26	296±4
Sudan grass (*Andropogon sorghum aethiopicus*), first crop.	June 30 to July 26.	337	108.1		49.4			457
		338	92 3		42.7			463
		339	90.2		42.5			471
		340	92.3		42.4			459
		341	106.6		41.0			385
		342	105 0		43.5			414
Mean								442±11
Sudan grass, second crop.	July 26 to Sept. 2.	337	67.6		27.7			410
		338	68.9		25.9			376
		339	59.0		22.3			378
		340	62.8		25.6			408
		341	54 2		23.2			428
		342	62.5		27 0			432
Mean								405±7
Sudan grass, combined crop.	June 30 to Sept. 2.	337	175.7		77.1			439
		338	161.2		68.6			426
		339	149 2		64.8			434
		340	155.1		68.0			438
		341	160.8		64.2			399
		342	167 5		70.5			421
Mean								426±3
Millet, Kursk, S. P I. 34771 (*Chaetochloa italica*).	June 17 to Aug. 12.	253	238.2	72.2	89.7	30	1242	377
		254	179.0	48.4	68.0	27	1405	380
		255	174.9	46.0	64.6	26	1404	369
		256	155.1	39.4	53.2	25	1350	343
		257	145.0	45.9	54.2	32	1181	374
		258	133.6	46.9	47.8	35	1019	358
Mean							1267±45	367±4

a Not included in the mean.

The grain production of corn was very uncertain and is not especially significant. The water requirement, based on grain, was 3634 ± 19, or 76 per cent above that in 1915. The water requirement for sorghum based on grain production, was 853 ± 26, or 24 per cent less than in 1915. This result is remarkable and shows strikingly the ability of sorghum to maintain a low water requirement even during an exceptionally dry and hot season.

The water requirement of millet was 1267 ±45, or 91 per cent higher than in 1915. With the exception of sorghum the water requirement based on grain yield, of the crops included in this experiment, shows about the same response as does that based on total dry matter.

WATER REQUIREMENT OF VARIETIES OF FLAX, 1916

The experiments with flax were very unsatisfactory (Table 17). The hot dry season was very unfavorable and only a few pots survived. The Jalaun variety gave a water requirement of 1098 ± 10, or 61 per cent higher than in 1915, and Kashgar a water requirement

of 812 ± 0, or 43 per cent higher than in 1915. Flax did not respond to the dry, hot year to any greater extent than the other crops grown. These values, therefore, fall within the range of expectation.

TABLE 17.—*Water requirement of varieties of flax at Akron, Colo., 1916*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
FLAX								
(*Linum usitatissimum*)			*Grams*	*Grams*	*Kgms.*	*P. ct*		
Jalaun, S. P. I. 36566, Indian, C. I. 21.	June 26 to Aug 9..	172	7. 2	1. 4	7. 9	19	_____	1097
		173	13. 7	1. 6	15. 4	12	963	1124
		174	8. 2	_____	8. 8	_____	_____	1073
Mean_____								1098±10
Kashgar, S. P. I. 37719, C. I. 50-1.	June 26 to Sept. 2..	175	62. 8	7 5	51. 0	12	_____	812
		178	84. 5	16. 9	68. 6	20	_____	812
Mean_____								812±0
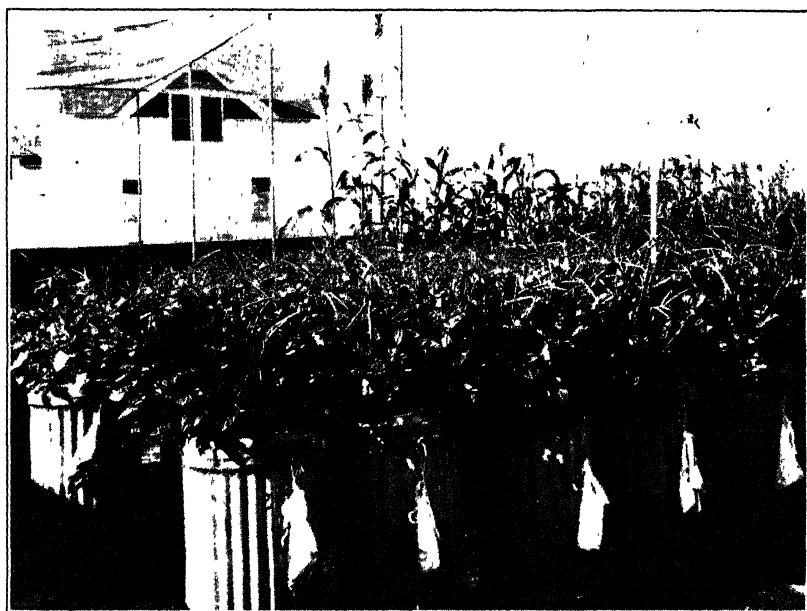
FIG. 5.—Cowpeas grown in the water requirement experiments at Akron, Colo., in 1916. Photographed August 2, 1916

WATER REQUIREMENT OF LEGUMES, 1916

The water requirement of alfalfa in 1916, 1047 ± 9, was higher than for any year with the exception of 1911. Alfalfa responds to dry and hot weather and the high water-requirement value in 1916 is due partly to the first or spring crop which gave a value almost as high as the second or midsummer crop. In 1916 the yield of three crops was less than the yield of two crops in 1915. The water requirement of alfalfa for 1916 was 51 per cent greater than for 1915.

Cowpeas (see fig. 5), although a southern crop, responded in a greater degree to the hot, dry season than any crop, excepting corn and oats. On the basis of total dry matter the water requirement was 767 ± 8, or 86 per cent higher than in 1915, and on the basis of seed production, 2373 ± 77, or 89 per cent above the 1915 results. Table 18 shows complete results for the 1916 water-requirement measurements of legumes.

TABLE 18.—*Water requirement, based on dry matter, of legumes and grain, at Akron, Colo., 1916*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
ALFALFA								
(*Medicago sativa*)			*Grams*	*Grams*	*Kgms.*	*P ct.*		
Grimm, A. D. I. E-23, first crop	June 24 to July 25__	199	18. 2	------	19. 7	-----	--------	1082
		200	20. 1	------	19. 1	-----	--------	950
		201	26. 5	------	30. 5	-----	--------	1151
		202	28. 0	------	30. 2	-----	--------	1079
		203	34. 0	------	45. 1	-----	--------	1326
		204	40. 5	------	48. 5	-----	--------	1198
Mean								1131±36
Grimm, A. D. I. E-23, second crop	July 25 to Aug. 30__	199	43. 8	------	52. 7	-----	--------	1203
		200	42. 2	------	55. 4	-----	--------	1313
		201	50. 0	------	57. 8	-----	--------	1156
		202	47. 1	------	56. 5	-----	--------	1200
		203	53. 1	------	61. 8	-----	--------	1164
		204	55. 9	------	66. 5	-----	--------	1190
Mean								1204±14
Grimm, A. D. I. E-23, third crop	Aug. 30 to Oct. 2__	199	41. 5	------	34. 7	-----	--------	836
		200	41. 1	------	34. 0	-----	--------	827
		201	42. 0	------	32. 0	-----	--------	762
		202	47. 4	------	38. 1	-----	--------	804
		203	50. 8	------	41. 0	-----	--------	807
		204	58. 0	------	53. 6	-----	--------	924
Mean								827±13
Grimm, A. D. I. E-23, combined crop	June 24 to Oct. 2__	199	103. 5	------	107. 1	-----	--------	1035
		200	103. 4	------	108. 5	-----	--------	1049
		201	118. 5	------	120. 3	-----	--------	1015
		202	122. 5	------	124. 8	-----	--------	1019
		203	137. 9	------	117. 9	-----	--------	1073
		204	154. 4	------	168. 6	-----	--------	1092
Mean								1047±9
COWPEA								
(*Vigna sinensis*)								
S. P. I. 29282	June 16 to Aug. 15_	211	145. 4	47. 9	106. 9	33	2232	735
		212	107. 9	32. 8	86. 6	30	2640	803
		213	107. 8	44. 8	85. 3	42	1904	791
		214	114. 5	34. 0	87. 9	30	2585	768
		215	115. 8	36. 1	86. 5	31	2396	747
		216	125. 2	38. 1	94. 5	30	2480	755
Mean							2373±77	767±8

WATER REQUIREMENT OF COTTON AND POTATOES, 1916

The water requirement of cotton was 612 ± 9 (Table 19), or 38 per cent above that of 1915. The hot, dry year was favorable for this crop, which was relatively as efficient in the use of water as sorghum, and was exceeded only by grama grass and Galgalos wheat.

TABLE 19.—*Water requirement of cotton, based on dry matter, and of potatoes, based on dry matter and tubers at Akron, Colo., 1916*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct*		
Cotton, Triumph (*Gossypium hirsutum*).	June 27 to Sept 29.	247	133.8	------	78.7	------	---------	588
		248	136.3	------	81 7	------	---------	599
		249	121.5	------	81.1	------	---------	667
		250	134.2	------	80 8	------	---------	602
		251	187.3	------	118 3	------	---------	632
		252	194.3	------	113.6	------	---------	585
Mean								612±9
POTATO								
(*Solanum tuberosum*)							*Tubers*	
McCormick	June 22 to Sept. 13.	187	120.7	27 6	90.2	23	3268	747
		188	98.3	14.3	82.9	15	5797	843
		189	108.1	26 9	92 7	25	3446	858
		190	110.5	2 4	68.1	22	2838	616
		191	74.1	.1	48.1	13	4810	649
		192	114.2	9.5	85.6	8	901	750
Mean							3510±452	744±28
Irish Cobbler	June 26 to Sept. 13.	193	109.8	55.3	61.4	50	1110	559
		195	127.4	90.9	82 5	71	908	648
		196	119.0	88.7	75.0	75	846	630
		197	133.7	100.4	79.9	75	796	598
		198	91.9	54.2	65.6	59	1210	714
Mean							974±63	630±17

Potatoes required about the same amount of water as in 1913. The McCormick variety was grown only in 1913 and 1916, both of which were dry and hot. Based on dry matter this variety required 744 ± 28, or 18 per cent more water than Irish Cobbler and on tuber production more than 3½ times as much. Irish Cobbler was relatively less efficient during the hot year, 1916, than the cool year, 1915, the water requirement being 630 ± 17 or 91 per cent above that of 1915. On the basis of tuber production, however, Irish Cobbler required only 974 ± 63, or 3 per cent more water than in 1915.

WATER REQUIREMENT OF NATIVE PLANTS, 1916

The most efficient plant was grama grass with a water requirement of 336 ± 8, the average for two crops, followed closely by pigweed with a water requirement for two crops of 340 ± 13 (Table 20). These values are low and were exceeded in 1916 by sorghum only. During the unusually hot, dry year grama grass showed an exceptional ability to efficiently utilize the water supply. Its relative water-requirement value, 21 points below the average of all crops (Table 28), was the lowest of any of the plants measured. Sorghum, the second best crop in relative water requirement, was 11 points above grama grass. Both plants showed unusually low values as compared with the results of other years. The water requirement of lamb's-quarters was 666 ± 27, of sunflower, 579 ± 10, and of Iva, 652 ± 6, or about equal to such crops as the small grains, but much higher than sorghum, corn, and millet.

TABLE 20.—*Water requirement, based on dry matter, of native plants, at Akron, Colo., 1916*

Kind of plant	Period of growth	Pot No	Dry matter	Water	Water requirements based on dry matter
GRAMA GRASS			*Grams*	*Kgms.*	
(*Bouteloua gracilis*)					
First crop	June 16 to July 26	223	15. 0	6. 1	407
		225	15. 7	6. 8	433
		226	12. 6	5. 1	405
		227	27. 6	11. 8	428
		228	16. 7	8. 0	479
Mean					430±9
Second crop	July 26 to Sept. 1	223	7. 9	1. 3	165
		225	17. 8	3. 9	219
		226	14. 7	3. 6	245
		227	31. 4	9. 2	293
		228	11 8	2 4	203
Mean					225±15
Combined crop	June 16 to Sept. 1	223	22 9	7 4	323
		225	33. 5	10 7	319
		226	27. 3	8. 7	319
		227	59. 0	21. 0	356
		228	28. 5	10. 4	365
Mean					336±8
PIGWEED					
(*Amaranthus retroflexus*)					
First crop	June 27 to July 26	205	67. 9	28 0	412
		206	62 2	28. 5	458
		207	58. 3	26. 4	453
		208	65. 0	30. 3	466
		209	58. 1	26 5	456
		210	17. 6	6. 9	392
Mean					440±9
Second crop	July 26 to Sept. 1	205	62. 6	14. 4	230
		206	56. 2	14. 9	265
		207	49 5	13. 5	273
		208	57. 6	14. 1	245
		209	57. 9	16 1	278
		210	52. 8	10. 8	205
Mean					249±9
Combined crop	June 27 to Sept 1	205	130. 5	42. 4	325
		206	118. 4	43. 4	367
		207	107. 8	39. 9	370
		208	122. 6	44 4	362
		209	116. 0	42. 6	367
		210	70. 4	17. 7	251
Mean					340±13
MISCELLANEOUS					
Lamb's-quarters (*Chenopodium album*)	July 13 to Sept. 1	37	97. 7	84. 2	862
		38	131. 1	84. 9	648
		39	135. 9	84. 4	621
		40	118. 8	81 1	683
		41	139. 0	87. 5	629
		42	146. 9	81. 5	555
Mean					666±27
Sunflower (*Helianthus annuus*)	July 13 to Sept. 4	229	256. 9	140. 0	545
		230	235. 2	132. 5	563
		231	218. 9	126. 8	579
		232	229. 4	140. 4	612
		233	216. 7	119. 7	552
		234	203. 7	127. 4	625
Mean					579±10
Iva xanthifolia	July 13 to Sept. 4	235	185 7	119. 9	646
		236	202. 9	129. 1	636
		237	173 6	110. 9	639
		238	115. 7	80. 2	693
		239	100. 9	64. 7	641
		240	214. 6	141. 4	659
Mean					652±6

It is especially interesting to note the relative behavior of different crops during this year of extreme climatic conditions. (See Tables 27, 28, 31, and 32.) The weather during the year was very hot and dry. The evaporation for the six-months' growing period, 47.18 inches, was the highest for any year from 1911 to 1917, with the exception of 1911. The precipitation, 10.77 inches, was also low.

As compared with 1911, the water-requirement values in 1916 are higher. This is probably due partly to the very high evaporation during July, 1916, the evaporation for that month being over 11 inches, or higher than the monthly record for the period 1908 to 1925, inclusive. This high value is reflected in the water-requirement measurements, since all crops were active during this period.

In Table 28 the relative values, based on actual values given in Table 27, afford a means of judging the relative efficiency or inefficiency in the use of water of any one of the crops grown for the different periods. The longest series of experiments were conducted during the years 1914–1917. The water requirement in 1916 was 22 points above the average for the period. Seven of the crops gave relative values less than 22 points above the average while eight gave values more than 22 points above. The highest value for the period was 37 points above the average and the lowest 21 points below.

Grama grass, with a water requirement 21 points below the average of the crops shown in Table 28, made the best individual crop record. Its remarkable adjustment to the hot, dry weather conditions during the year stamps grama grass as relatively efficient in its use of water. On the basis of grain production sorghum, with a water requirement 51 points below the average of crops shown in Table 29, made an exceptionally good showing. Cotton has a value 8 points below and Galgalos wheat 9 points below the mean for all crops. The hot year reacted most unfavorably on Swedish Select oats which was 15 points above the average, followed by Northwestern Dent corn, cowpeas, Burt oats, and Kursk millet, 13, 10, 7, and 6 points, respectively, above the average.

The water requirement on the basis of grain production was higher in 1916 than in any other year. Sorghum showed the lowest relative water requirement while Northwestern Dent corn showed the highest.

WATER REQUIREMENT OF PLANTS, 1917

Only 16 sets of plants were used in the regular water-requirement experiments during 1917. These included crops previously grown. The climatic conditions during the year were about normal; and the evaporation was about the average for the entire observation period. The water requirement of the seven crops grown during the period, 1911–1917, averaged about the same as for the entire period. The season was similar to 1913 and 1914, although the rainfall was greater.

WATER REQUIREMENT OF WHEAT, OATS, BARLEY, AND RYE, 1917

Only two varieties of wheat were grown in the regular series in 1917. Kubanka had a water requirement of 471 ± 4 and Galgalos of 543 ± 6 (Table 21). The oats varieties, Swedish Select and Burt, had practically the same values, 635 ± 5 and 636 ± 5, respectively.

The water requirement of rye, 625 ± 5, was a little less than for oats and barley, 522 ± 4, and a little lower than Galgalos wheat. Based on index values, Kubanka and Galgalos wheat had a water requirement in 1917 considerably below the mean of the series. The oats varieties, as well as those of barley and rye, on the other hand, required proportionately more water than wheat.

TABLE 21.—*Water requirement, on the basis of grain and dry matter, of wheat, oats, barley, and rye, at Akron, Colo., 1917*

Kind of plant	Period of growth	Pot No	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Wheat, Kubanka, C. I. 1440 (*Triticum durum*).	June 11 to Aug 21.	1	145.7	51.2	71.3	35	1393	489
		2	161.7	52.0	77.5	32	1490	479
		3	149.4	51.3	71.0	34	1384	475
		4	165.1	58.5	75.9	35	1297	460
		5	131.9	46.1	61.1	35	1325	463
		6	164.6	57.4	75.3	35	1312	457
Mean							1367±21	471±4
Wheat, Galgalos, C. I. 2398 (*T. aestivum*)	June 11 to Aug. 17.	7	159.3	55.5	87.6	35	1578	550
		8	156.9	57.0	85.9	36	1507	547
		9	150.9	53.3	80.0	35	1501	530
		10	164.4	61.4	88.1	37	1435	536
		11	169.0	59.8	87.7	35	1467	519
		12	158.1	56.7	91.0	36	1605	576
Mean							1516±19	543±6
Oats, Swedish Select, C. I. 134 (*Avena sativa*).	June 11 to Aug. 17.	13	185.6	53.9	115.0	29	2134	620
		14	169.4	54.5	105.0	32	1927	620
		15	161.3	48.3	105.1	30	2176	652
		16	161.6	49.3	102.4	31	2077	634
		17	194.9	57.2	128.7	29	2250	660
		18	152.7	48.3	94.9	32	1965	621
Mean							2088±37	635±5
Oats, Burt, C. I. 293 (*A. sativa*).	June 11 to Aug. 6.	19	150.0	42.5	99.3	28	2336	662
		20	146.3	53.8	94.5	37	1757	646
		21	151.5	46.2	96.7	31	2093	638
		22	162.2	59.9	103.4	37	1726	637
		23	164.5	71.7	102.1	44	1424	621
		24	148.3	50.7	90.6	34	1787	611
Mean							1854±91	636±5
Barley, Hannchen, C. I. 531 (*Hordeum distichon*).	June 11 to Aug. 15.	25	114.7	49.8	58.6	43	1177	511
		26	136.0	63.1	68.9	46	1092	507
		27	152.3	68.0	81.1	45	1193	533
		28	143.1	66.8	75.3	47	1127	526
		29	148.8	70.7	79.7	48	1127	536
		30	142.9	63.3	74.1	44	1171	519
Mean							1148±12	522±4
Rye, Vern, C. I. 73 (*Secale cereale*).	June 11 to Aug. 22.	31	123.2	35.7	78.0	29	2185	633
		32	133.8	50.6	84.3	38	1666	630
		33	101.7	34.7	61.2	34	1764	602
		34	104.7	33.3	64.7	32	1943	618
		35	131.9	42.6	80.5	32	1890	610
		36	131.8	35.3	86.2	27	2442	654
Mean							1982±84	625±5

A number of wheats were grown in the field in pots 6 feet long, holding the same amount of soil as the standard pots. These crops grew on the stored soil moisture. The results of this test are given in Table 22. The values of the wheats grown in the field should be

reduced by 10 per cent, to compare with those in the shelter. This reduced value has been used in the discussion and in the summary table. The water requirements of the wheat crops are as follows:

Durum wheats:
    Kubanka, C. I. 1440_____ 471±4
    C. I. 4082_____ 465±11
                            ─────────
    Average_____ 468±8

Common:
    Power, C. I. 3697_____ 459±8
    Glyndon, C. I. 2873_____ 472±4
    Ghirka_____ 473±57

Common—Continued.
    Prelude_____ 482±25
    Pioneer_____ ᵃ490
    Haynes Bluestem_____ 515±6
    Galgalos_____ 543±6

    Average_____ 491±26

    Average for the series_ 486±23

The durum wheats are a little more efficient than the common wheats. The most efficient is Kubanka, with a water requirement of 471±4, and the least efficient is Galgalos with a water requirement of 543±6, 15 per cent above Kubanka.

TABLE 22.—*Water requirement, based on grain and dry matter, of wheat varieties in 6-foot cans, at Akron, Colo., 1917*

Kind of plant	Period of growth	Pot No	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Haynes' Bluestem (*Triticum aestivum*).	June 9 to Sept. 12__	5	32.6	11.4	18.4	35	1614	564
		6	21.9	4.1	12.7	19	3098	580
Mean_____								572±7
C. I. 4082, from Peru (*T. durum*).	June 9 to Sept. 12__	7	20.0		9.8			490
		8	15.4	2.8	7.9	18	2821	513
		9	16.8	3.4	9.2	20	2706	548
Mean___ _____								517±12
Glyndon, C. I. 2873 (*T. aestivum*).	June 9 to Sept. 12__	13	10.8	3.2	5.7	30	1781	528
		14	23.8	6.1	12.2	26	2000	513
		15	14.7	3.6	7.8	24	2167	531
Mean_____								524±4
Power, C. I. 3697 (*T. aestivum*).	June 9 to Sept. 12__	16	17.8		8.7			489
		17	17.3	2.8	8.9	16	3179	514
		18	19.5		10.3			528
Mean_____								510±9
Ghirka (*T. aestivum*)_____	June 9 to Sept. 12__	19	15.8	2.9	9.5	18	3276	601
		20	12.2		5.5			451
Mean_____								526±63
Prelude, C. I. 4323 (*T. aestivum*).	June 9 to Aug. 22__	25	14.7		7.9			537
		26	7.6	3.1	4.6	41	1484	605
		27	8.6		4.0			465
Mean_____								536±28
Pioneer (*T. aestivum*)_____	June 9 to Aug. 28__	28	9.0		4.9			544

ᵃ Omitted in estimating probable error of means.

WATER REQUIREMENT OF CORN, SORGHUM, SUDAN GRASS, AND MILLET, 1917

One variety of each of the crops was grown in 1917, the water-requirement values being as follows:

Sorghum, Minnesota Amber___ 272±5   Corn, Northwestern Dent_____ 346±3
Millet, Kursk_____ 284±5   Sudan grass_____ 378±3

Although it had the lowest actual water requirement, sorghum was 6 points above the average for all crops on the basis of the index values, and of the crops here considered was exceeded only by Sudan grass, which was 7 points above the average of all crops for the year. Millet and corn departed only two points from the average. Climatically, 1917 was about an average year. Table 23 gives the results of the measurements of these four crops in detail.

TABLE 23.—*Water requirement, based on grain and dry matter, of corn, sorghum, sudan grass, and millet, at Akron, Colo., 1917*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms*	*P. ct*		
Corn, Northwestern Dent, (*Zea mays*)	June 14 to Sept. 1	67	363.3	59.2	120.0	16	2027	330
		68	330.7	66.6	116.2	20	1745	351
		69	333.8	13.4	115.6	1	a 8627	346
		70	343.5	11.6	124.5	3	a 10733	362
		71	350.6	59.7	120.3	17	2015	343
		72	299.0	6.5	126.1	2	a 19400	a 422
Mean							1929±73	346±3
Sorghum, Black Amber, Minnesota 341-13 (*Andropogon sorghum*).	June 20 to Sept. 4	61	320.8	56.6	81.2	18	1435	253
		62	285.1	22.1	82.9	8	a 3751	291
		63	262.1	31.9	72.6	12	2276	277
		64	224.5	12.9	68.7	6	a 5326	a 306
		65	269.4	34.7	70.9	13	2013	263
		66	295.1	77.7	81.1	26	1014	275
Mean							1700±224	272±5
Millet, Kursk, S. P. I. 34771 (*Chaetochloa italica*).	June 20 to July 30	37	199.1		59.3			298
		38	235.6		65.5			278
		39	231.9		62.9			271
		40	203.8		61.8			303
		41	277.5		72.1			260
		42	227.1		66.9			295
Mean								284±5
SUDAN GRASS (*Andropogon sorghum aethiopicus*)								
S. P. I. 25017, first crop	June 20 to Aug. 2	79	145.9		55.5			380
		80	139.6		57.0			408
		81	141.7		58.2			411
		82	143.6		56.8			396
		83	134.9		56.7			420
		84	138.8		59.3			427
Mean								407±5
S. P. I. 25017, second crop	Aug. 2 to Sept. 10	79	108.3		36.5			337
		80	95.7		32.5			340
		81	106.8		36.1			338
		82	104.1		34.6			332
		83	104.5		37.0			354
		84	113.8		37.7			331
Mean								339±2
S. P. I. 25017, combined crop.	June 20 to Sept. 10	79	254.2		92.0			362
		80	235.3		89.5			380
		81	248.5		94.3			379
		82	247.7		91.4			369
		83	239.4		93.7			391
		84	252.6		97.0			384
Mean								378±3

a Omitted in the mean.

WATER REQUIREMENT OF ALFALFA, 1917

The water requirement for three crops of alfalfa 822 ± 8 (Table 24) was the lowest value for the entire period except the damp cool years 1912 and 1915. Alfalfa seems to have been a little more efficient in its use of water during 1917 than during the period, 1911–1917.

TABLE 24.—*Water requirement, on the basis of dry matter, of alfalfa, at Akron, Colo., 1917*

Kind of plant	Period of growth	Pot No.	Dry matter	Water	Water requirements based on dry matter
ALFALFA					
(*Medicago sativa*)			*Grams*	*Kgms.*	
Dillman, E–23, first crop	June 22 to Aug. 3	55	60. 3	59. 8	992
		56	59. 0	56 5	958
		57	54. 6	40. 3	738
		58	54. 6	44. 4	813
		59	59. 3	54. 4	917
		60	52. 8	47 7	903
Mean					887±28
Dillman, E–23, second crop	Aug 3 to Sept. 3	55	71 3	47. 0	659
		56	63. 3	47. 4	749
		57	46. 8	43. 4	927
		58	50. 5	45. 3	897
		59	57. 7	47. 1	816
		60	49. 8	45. 9	922
Mean					828±33
Dillman, E–23, third crop	Sept. 3 to Oct. 9	55	79. 1	57 2	723
		56	72 9	54. 6	749
		57	52. 6	47. 9	911
		58	70. 2	53. 2	758
		59	75. 4	57. 0	756
		60	70. 8	53. 5	756
Mean					776±17
Dillman, E–23, combined crop	June 22 to Oct. 9	55	210. 7	164. 0	778
		56	195. 2	158. 5	812
		57	154. 0	131. 6	855
		58	175. 3	142 9	815
		59	192. 4	158. 5	824
		60	173. 4	147. 1	848
Mean					822±8

WATER REQUIREMENT OF COTTON, FLAX, AND COWPEAS, 1917

On the basis of index values, cowpeas, with a water requirement of 481 ± 4 (Table 25), was 14 points below the average. Cotton responded about as would be expected in view of the weather conditions, giving a water requirement value near the mean of all crops for the year.

TABLE 25.—*Water requirement, based on dry matter, of cotton, flax, and cowpeas, at Akron, Colo., 1917*

Kind of plant	Period of growth	Pot No.	Dry matter	Grain	Water	Grain	Water requirements based on—	
							Grain	Dry matter
			Grams	*Grams*	*Kgms.*	*P. ct.*		
Cotton, Triumph (*Gossypium hirsutum*).	June 29 to Oct. 8..	73	294.5	-------	149.0	-------	-------	506
		74	300.5	-------	156.9	-------	-------	522
		75	300.7	-------	151.7	-------	-------	504
		76	229.6	-------	119.4	-------	-------	520
		77	287.8	-------	154.1	-------	-------	535
		78	237.4	-------	128.8	-------	-------	543
Mean								522±4
Flax, Damont, C.I.3(*Linum usitatissimum*).	June 22 to Sept. 7..	97	34.7	6.9	20.1	-------	-------	579
		99	35.1	5.0	21.0	-------	-------	598
		100	26.5	2.6	15.0	-------	-------	566
		101	20.5	2.6	12.3	-------	-------	600
		102	19.2	2.4	13.1	-------	-------	682
Mean								605±13
Cowpea, S.P.I.29282 (*Vigna sinensis*).	June 20 to Aug. 30.	43	161.1	70.4	75.9	44	1078	471
		44	169.3	71.1	84.5	42	1188	499
		45	170.1	70.9	78.8	42	1111	463
		46	161.8	69.6	77.0	43	1106	476
		47	174.9	73.2	84.7	42	1157	484
		48	183.3	73.6	89.9	40	1221	490
Mean							1144±17	481±4

WATER REQUIREMENT OF GRAMA GRASS AND PIGWEED, 1917

Grama grass, whose water requirement was 290 ± 9 (Table 26) had a relative value 10 points below the average of all crops in 1917 and a lower actual value than for any particular year of the entire period of the experiments. Pigweed, with a water requirement of 307 ± 6, however, was 7 points above the average of all crops on the basis of index figures.

TABLE 26.—*Water requirement, based on dry matter, of grama grass and pigweed at Akron, Colo., 1917*

Kind of plant	Period of growth	Pot No.	Dry matter	Water	Water requirements based on dry matter
GRAMA GRASS (*Bouteloua gracilis*)			*Grams*	*Kgms.*	
First crop	June 22 to Aug. 20.	91	41.0	10.5	256
		92	48.3	13.0	269
		93	28.7	6.8	237
		94	43.3	12.8	296
		95	13.3	4.6	346
		96	11.4	3.6	316
Mean					287±12
Second crop	Aug. 20 to Oct. 12.	91	9.8	3.5	357
		92	9.4	2.2	234
		93	9.4	3.0	319
		94	11.3	3.1	274
		95	6.3	2.5	397
		96	7.0	1.7	243
Mean					304±20
Combined crop	June 22 to Oct. 12.	91	50.8	14.0	276
		92	57.7	15.2	263
		93	38.1	9.8	257
		94	54.6	15.9	291
		95	19.6	7.1	362
		96	18.4	5.3	288
Mean					290±9

TABLE 26.—*Water requirement, based on dry matter, of grama grass and pigweed, at Akron, Colo., 1917*—Continued

Kind of plant	Period of growth	Pot No.	Dry matter	Water	Water requirements based on dry matter
PIGWEED (*Amaranthus retroflexus*)			*Grams*	*Kgms.*	
First crop	June 20 to Aug. 3	49	119. 5	40. 4	338
		50	24. 2	10. 2	421
		51	42. 4	15. 4	363
		52	120. 0	40 8	340
		53	111. 4	37. 3	335
		54	128. 5	42. 2	328
Mean					354±10
Second crop	Aug. 3 to Sept. 1	49	30. 3	6. 5	215
		50	11. 3	2. 1	186
		51	50. 3	9. 5	189
		52	35. 9	7. 2	201
		53	36. 3	7. 3	201
		54	30. 8	5. 7	185
Mean					196±4
Combined crop	June 20 to Sept. 1	49	149. 8	46. 9	313
		50	35. 5	12. 3	346
		51	92. 7	24. 9	269
		52	155. 9	48. 0	308
		53	147. 7	44. 6	302
		54	159. 3	47. 9	301
Mean					307±6

SUMMARY OF WATER-REQUIREMENT MEASUREMENTS MADE IN 1917

Although the evaporation in 1917 was slightly above normal, the water requirement of all crops was slightly below normal. (See Table 28.) The only crops to show a water requirement above the average for all years were: Pigweed, Sudan grass, sorghum, and Burt oats, with water requirements, 7, 7, 6, and 5 points, respectively, above the average for the year.

Cowpeas, grama grass, Kubanka wheat, Galgalos wheat, alfalfa, and corn, with water requirements 14, 10, 4, 3, 2, and 2 points, respectively, below the average for the year, were also below the average for the entire experimental period. Cowpeas and grama grass were especially efficient, while pigweed and sorghum, crops of about the same temperature requirements, were relatively inefficient.

GENERAL SUMMARY OF WATER-REQUIREMENT MEASUREMENTS MADE AT AKRON, COLO.

For the purpose of comparing the water requirement of the series for different years the results with those crops grown continuously during the period (see Table 27) have been reduced to a percentage basis (Table 28). In this way the over-emphasis which would otherwise be placed on plants with a high water requirement is avoided. If the water requirement results for 1911 are compared with those of the average of the series (see Table 27), a mean result 8 per cent above the average of the series if based on actual values, and 7 per cent above if based on percentages is obtained (see Table 28). This difference is the result of averaging such divergent values as 1,068 for alfalfa and 298 for sorghum. The use of the actual averages in this case would be equivalent to weighting the results in proportion to their values.

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TABLE 27.—*The water requirement, based on dry matter, of crops grown at Akron, Colo., for the periods 1911–1917, 1912–1917, 1913–1917, and 1914–1917, inclusive, and the evaporation in inches for the period, April to September, inclusive, of each year*

Crop	Seasonal water requirement and evaporation							Average
	1911	1912	1913	1914	1915	1916	1917	
PERIOD 1911–1917								
Alfalfa, A. D. I. E-23	1038±16	657±11	834±8	890±6	695±9	1047±9	822±8	859±10
Oats, Swedish Select, C. I. 134	615±7	423±5	617±9	599±2	448±10	876±21	635±5	602±11
Oats, Burt, C. I. 293	639±7	449±3	617±5	615±6	445±5	809±5	636±5	601±5
Barley, Hannchen, C. I. 531	527±8	443±3	ª513±12	501±5	404±11	664±9	522±4	511±8
Wheat, Kubanka, C. I. 1440	468±8	394±7	496±5	518±6	405±3	636±14	471±4	484±7
Corn, Northwestern Dent	368±10	280±10	399±12	368±6	253±7	495±13	346±3	358±9
Millet, Kursk, S. P. I. 34771	ᵇ287±2	187±2	286±4	295±2	202±1	367±4	284±5	273±3
Sorghum, Minnesota Amber, A. D. I. 341–13	ᶜ298±4	239±2	298±2	284±3	203±3	296±4	272±5	270±3
Average	534±9	384±6	508±8	509±5	382±7	649±11	499±5	495±8
Evaporation in inches, April to September	48. 80	37. 75	43. 06	41. 86	33. 40	47. 17	42. 70	42. 11
PERIOD 1912–1917								
Alfalfa, A. D. I. E-23		657±11	834±8	890±6	695±9	1017±9	822±8	824±9
Oats, Swedish Select, C. I. 134		423±5	617±9	599±2	448±10	876±21	635±5	600±11
Oats, Burt, C. I. 293		449±3	617±5	615±6	445±5	809±5	636±5	595±5
Barley, Hannchen, C. I. 531		443±3	ª513±12	501±5	404±11	664±9	522±4	508±8
Wheat, Kubanka, C. I. 1440		394±7	496±5	518±6	405±3	636±14	471±4	487±7
Corn, Northwestern Dent		280±10	399±12	368±6	253±7	495±13	346±3	357±9
Millet, Kursk, S. P. I. 34771		187±2	286±4	295±2	202±1	367±4	284±5	270±3
Sorghum, Minnesota Amber, A. D. I. 341–13		239±2	298±2	284±3	203±3	296±4	272±5	265±3
Cotton, Triumph		488±14	657±11	574±9	443±8	612±9	522±4	549±10
Average		396±8	524±8	516±5	389±7	645±11	501±5	495±8
Evaporation in inches, April to September		37. 75	43. 06	41. 86	33. 40	47. 17	42. 70	
PERIOD 1913–1917								
Alfalfa, A. D. I. E-23			834±8	890±6	695±9	1047±9	822±8	858±8
Oats, Swedish Select, C. I. 134			617±9	599±2	448±10	876±21	635±5	635±11
Oats, Burt, C. I. 293			617±5	615±6	445±5	809±5	636±5	624±5
Barley, Hannchen, C. I. 531			ª513±12	501±5	404±11	664±9	522±4	521±9
Wheat, Kubanka, C. I. 1440			496±5	518±6	405±3	636±14	471±4	505±7
Corn, Northwestern Dent			399±12	368±6	253±7	495±13	346±3	372±9
Millet, Kursk, S. P. I. 34771			286±4	295±2	202±1	367±4	284±5	287±3
Sorghum, Minnesota Amber, A. D. I. 341–13			298±2	284±3	203±3	296±4	272± 5	271±4
Cotton, Triumph			657±11	574±9	443±8	612±9	522±1	562±9
Cowpea, S. P. I 29282			571±3	659±5	413±5	767±8	481±4	578±5
Pigweed			320±7	306±1	229±3	310±13	307±6	300±7
Grama grass			ᵈ380±12	380±7	312±14	336±8	290±9	313±10
Average			500±8	500±5	371±8	604±11	466±5	488±8
Evaporation in inches, April to September			43. 06	41. 86	33. 40	47. 17	42. 70	
PERIOD 1914–1917								
Alfalfa, A. D. I E-23				890±6	695±9	1047±9	822±8	864±8
Oats, Swedish Select, C. I. 134				599±2	448±10	876±21	635±5	640±12
Oats, Burt, C. I. 293				615±6	445±5	809±5	636±5	626±5
Barley, Hannchen, C. I. 531				501±5	404±11	664±9	522±4	523±8
Wheat, Kubanka, C. I. 1440				518±6	405±3	636±14	471±4	508±8
Corn, Northwestern Dent				368±6	253±7	495±13	346±3	366±8
Millet, Kursk, S. P. I. 34771				295±2	202±1	367±4	284±5	287±3
Sorghum, Minnesota Amber, A. D. I 341–13				284±3	203±3	296±4	272±5	264±4
Cotton, Triumph				574±9	443±8	612±9	522±4	538±8
Cowpea, S. P. I. 29282				659±5	413±5	767±8	481±4	580±6
Pigweed				306±1	229±3	340±13	307±6	296±7
Grama grass				389±7	312±14	336±8	290±9	332±10
Rye, Vern, C. I. 73				622±7	469±8	800±11	625±5	629±8
Wheat, Galgalos, C. I. 2398				624±5	481±4	652±8	543±6	575±6
Sudan Grass, S. P. I. 25017				394±4	260±3	426±3	378±3	365±3
Average				509±5	377±7	608±10	476±5	493±7
Evaporation in inches, April to September				41. 86	33. 40	47. 17	42. 70	

ª Interpolated value   ᶜ Red Amber Sorghum.
ᵇ S. P. I. 22420.   ᵈ Bouteloua gracilis and B. dactyloides mixed.

TABLE 28.—*Water requirement and evaporation results shown in Table 27 expressed in index numbers of the mean value for each crop, taken as 100; and the ratio of the water requirement index to the evaporation index*

Crop	Index numbers of water requirement and evaporation, and water requirement-evaporation ratio index numbers of different crops.							Mean
	1911	1912	1913	1914	1915	1916	1917	
PERIOD 1911–1917								
Alfalfa, A. D. I. E-23	124	76	97	104	81	122	96	100
Oats, Swedish Select	102	70	102	100	74	146	105	100
Oats, Burt	106	75	103	102	74	135	106	100
Barley, Hannchen	103	87	100	98	79	130	102	100
Wheat, Kubanka, C. I 1440	97	81	102	107	84	131	97	100
Corn, Northwestern Dent	103	78	111	103	71	138	97	100
Millet, Kursk	105	68	105	108	74	134	104	100
Sorghum, Minnesota Amber	110	89	110	105	75	110	101	100
Water requirement average	107	78	104	103	77	131	101	100
Evaporation average	116	90	102	99	79	112	101	100
W/E	92	87	102	104	97	117	100	100
PERIOD 1912–1917								
Alfalfa, A. D. I. E-23		80	101	108	84	127	100	100
Oats, Swedish Select		71	103	100	75	146	106	100
Oats, Burt, C. I. 293		75	104	103	75	136	107	100
Barley, Hannchen		87	101	99	80	131	103	100
Wheat, Kubanka, C. I. 1440		81	102	106	83	131	97	100
Corn, Northwestern Dent		78	112	103	71	139	97	100
Millet, Kurst		69	106	109	75	136	105	100
Sorghum, Minnesota Amber		90	112	107	77	112	103	100
Cotton, Triumph		89	120	105	81	111	95	100
Water requirement average		80	107	104	78	130	101	100
Evaporation average		93	105	102	81	115	104	100
W/E		86	102	102	96	113	97	100
PERIOD 1913–1917								
Alfalfa, A. D. I. E-23			97	104	81	122	96	100
Oats, Swedish Select			97	94	71	138	100	100
Oats, Burt, C. I. 293			99	99	71	130	102	100
Barley, Hannchen			98	96	78	127	100	100
Wheat, Kubanka, C. I. 1440			98	103	80	126	93	100
Corn, Northwestern Dent			107	99	68	133	93	100
Millet, Kursk			100	103	70	128	99	100
Sorghum, Minnesota Amber			110	105	75	109	100	100
Cotton, Triumph			117	102	79	109	93	100
Cowpea, S. P. I 29282			99	114	71	133	83	100
Pigweed			107	102	76	113	102	100
Grama grass			113	113	91	98	85	100
Water requirement average			104	103	76	122	96	100
Evaporation average			103	101	80	113	103	100
W/E			101	102	95	108	93	100
PERIOD 1914–1917								
Alfalfa, A. D. I. E-23				103	81	121	95	100
Oats, Swedish Select				94	70	137	99	100
Oats, Burt				98	71	129	102	100
Barley, Hannchen				96	77	127	100	100
Wheat, Kubanka, C. I. 1440				102	80	125	93	100
Corn, Northwestern Dent				101	69	135	95	100
Millet, Kursk				103	70	128	99	100
Sorghum, Minnesota Amber				108	77	112	103	100
Cotton, Triumph				107	82	114	97	100
Cowpea				114	71	132	83	100
Pigweed				103	77	115	104	100
Grama grass				117	94	101	87	100
Rye, Vern				99	75	127	99	100
Wheat, Galgalos				109	84	113	94	100
Sudan grass				108	71	117	104	100
Water requirement average				104	77	122	97	100
Evaporation average				101	81	114	103	100
W/E				103	95	107	94	
Index averages used	107	80	104	104	77	122	97	

TABLE 29.—*Water-requirement values (actual and index) based on grain of all crops grown at Akron, Colo., for the periods 1911–1917, inclusive*

WATER REQUIREMENT (ACTUAL VALUES) FOR CROPS

Crop	1911	1912	1913	1914	1915	1916	1917	Average
Oats, Swedish Select	1632±35	1103±18	1876±55	1421±8	1102±34	2288±39	2088±37	1641±35
Oats, Burt	1500±57	1224±55	1641±33	1483±31	1150±27	1975±49	1854±91	1547±53
Wheat, Kubanka, C I 1440	1191±14	1111±37	1322±16	1367±13	1232±13	1779±108	1367±21	1338±45
Corn, Northwestern Dent	2040±342	954±106	1241±77	a1846±159	2060±108	3634±19	1929±73	1958±159
Millet, Kursk	b 923±40	483±11	985±99	1075±38	665±24	1267±45	a1085±74	926±55
Sorghum, Minnesota Amber	c1494±202	607±15	765±12	893±26	1116±105	853±26	1700±224	1061±122
Average	1463±165	914±52	1305±58	1348±69	1221±65	1966±56	1671±109	1412±91
Average for first 3 crops	1441±39	1146±40	1613±38	1424±20	1161±26	2014±72	1770±58	1510±45

WATER REQUIREMENT (INDEX VALUES) FOR CROPS

Crop	1911	1912	1913	1914	1915	1916	1917	Average
Oats, Swedish Select	99	67	114	86	67	139	127	100
Oats, Burt	97	79	106	96	74	128	120	100
Wheat, C. I. 1440	89	83	99	102	92	133	102	100
Corn, Northwestern Dent	104	49	63	94	105	186	99	100
Millet, Kursk	100	52	106	116	72	137	117	100
Sorghum, Minnesota Amber	141	57	72	84	105	80	160	100
Average	105	65	93	96	86	134	121	100
Average for first 3 crops	95	76	106	95	78	133	116	100

WATER REQUIREMENT (ACTUAL VALUES) FOR CROPS

Crop	1911	1912	1913	1914	1915	1916	1917	Average
Oats, Swedish Select				1421±8	1102±34	2288±39	2088±37	1725±32
Oats, Burt				1483±31	1150±27	1975±49	1854±91	1616±56
Wheat, Kubanka, C. I. 1440				1367±13	1232±13	1779±108	1367±21	1436±56
Wheat, Galgalos				1900±30	1551±65	2445±155	1516±19	1853±86
Rye, Vern				2291±54	1485±80	2871±99	1982±84	2157±81
Barley, Hannchen				1179±28	919±50	1425±27	1148±12	1175±32
Corn, Northwestern Dent				1846±159	2060±108	3634±19	1929±73	2367±103
Sorghum, Minnesota Amber				893±26	1116±105	853±26	1700±224	1111±125
Millet, Kursk				1075±38	665±24	1267±45	1085±74	1023±49
Cowpea				1962±27	1257±39	2373±77	1144±17	1684±46
Average				1542±58	1257±63	2091±77	1581±89	1618±73

WATER REQUIREMENT (INDEX VALUES) FOR CROPS

Crop	1911	1912	1913	1914	1915	1916	1917	Average
Oats, Swedish Select				82	64	133	121	100
Oats, Burt				92	71	122	115	100
Wheat, Kubanka, C. I. 1440				95	86	124	95	100
Wheat, Galgalos				103	84	132	82	100
Rye, Vern				106	69	133	92	100
Barley, Hannchen				100	81	121	98	100
Corn, Northwestern Dent				78	87	154	81	100
Sorghum, Minnesota Amber				78	98	75	149	100
Millet, Kursk				105	65	124	106	100
Cowpea				117	75	141	68	100
Average				96	78	126	101	100

a Interpolated on the basis of the ratio of the averages of the first three crops for the years 1911–1913 and 1915–1917 to the average for corn for the same years and the average of the first three crops to the interpolated values for corn for the year 1914. Interpolation for millet by the same method.

b S. P. I. 22420 used in 1911 instead of S. P. I. 34771.

c Red Amber used in 1911 instead of Minnesota Amber.

While the results are presented in percentages of the average, the basis is in each case 100, and the values may be regarded as index numbers. It is possible, then to express the relative water requirement of crops in terms of positions, of points above or points below the average. To express these relative positions as percentages above or below would lead to confusion. By the use of these simple index numbers, logarithms, which would have made the results more difficult for the general reader, have been dispensed with. The index averages at the bottom of Table 28 represent as nearly as possible the response of crop plants to the different seasons. Accepting these values, the actual values summarized in Table 34 have been weighted and averaged to give a column of weighted values, which represents the probable values if each crop had been grown each year of the experiment. This value assumes that the mean values of average index numbers hold for all crops. The greatest observed error in this assumption for the crops grown continuously for four or more years is 20 per cent. In Table 29 the water requirement based on grain production has been treated in the same way as the water requirement based on total dry matter in Tables 27 and 28. The index numbers based on grain production are also given in Table 29. The water requirement based on grain production is much more variable than when based on dry matter. The same index figures have been used, therefore, in computing the values in Table 34 as were used in computing those in Table 33.

EFFECT OF SEASON ON WATER REQUIREMENT

The values in Table 27 have been reduced in Table 28, to index numbers having a mean value of 100, and may be compared directly to determine the effect of the different years. While the values are high or low in proportion to the favorable or unfavorable season there are minor crop differences of great physiological significance. The variation in water requirement from year to year is greater proportionately than the variation in evaporation. The evaporation index numbers for the period 1911 to 1917, inclusive, varied from 79 points for 1915, to 116 points for 1911, a range of only 37 points, while the mean water requirement index numbers varied from 77 points for 1915, to 131 points for 1916, a range of 54 points. The greatest water requirement range for any single crop was from 70 to 146, or 76 points, for Swedish Select oats; and the smallest range was from 75 to 110, or 35 points, for sorghum.

Evaporation does not vary as much from year to year as does the water requirement. (See Tables 27 and 28.) This is to be expected, for during years of exceedingly unfavorable conditions when the evaporation is unusually high, plants respond by evaporating freely to the dry air and may even be injured by the high evaporation and temperature to the extent that the rate of growth is retarded. A disproportionately great effect on the water requirement would therefore be expected.

THE RESPONSE OF PLANTS TO DIFFERENT SEASONS

The effect of the seasons was much the same on all crops. (See figs. 6 and 7.) They responded in varying degrees, however, corn and oats being most responsive, and sorghum and grama grass least responsive. (See Table 28.)

On the basis of the behavior of all crops grown from 1911 to 1917, inclusive (see Table 28), the values for 1911 have two outstanding results—the water requirement of alfalfa was 17 points too high and
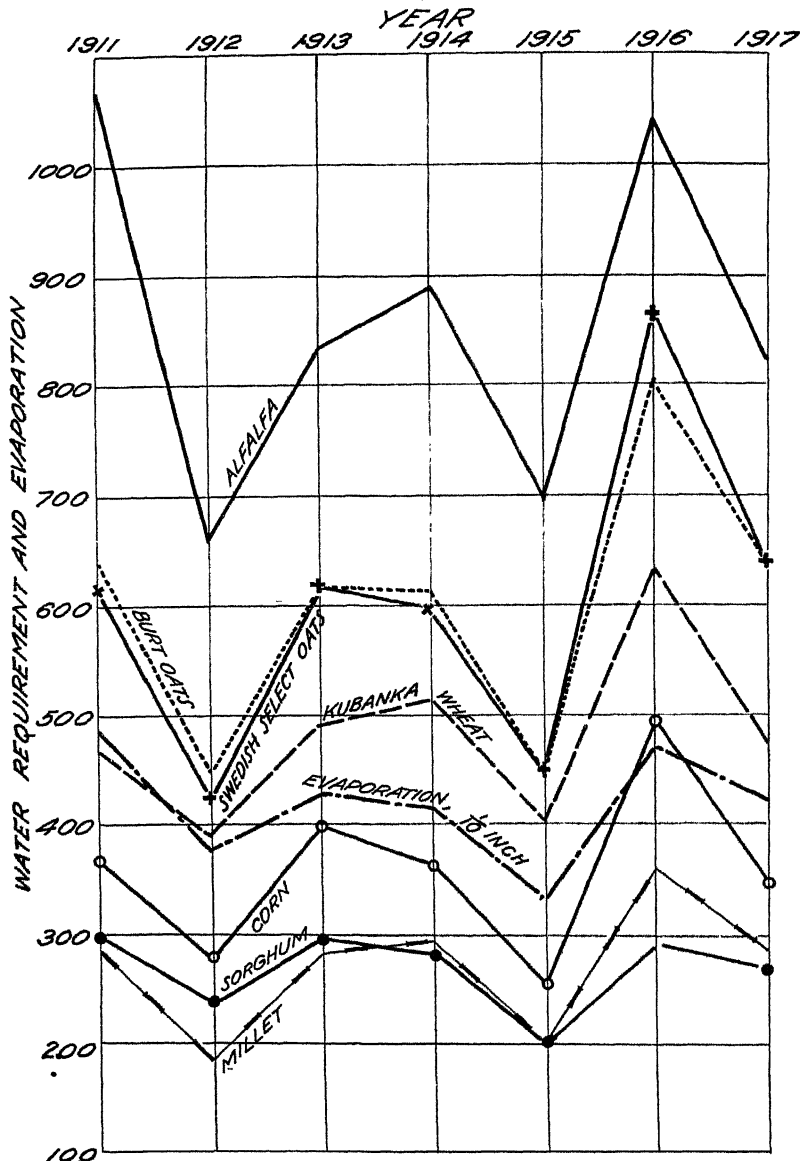
FIG. 6.—The water requirement of different crops and evaporation in tenths of an inch for the years 1911–1917

Kubanka wheat 10 points too low. No thoroughly satisfactory explanation can be made of these results. On the basis of total amount of growth, alfalfa was high and Kubanka wheat low. (See Table 30.)   The year 1911 was hot and dry (see Tables 31 and 32),

and Kubanka wheat is not affected by such conditions to the same extent as are many of the other crops. In 1912 the water requirement of Kubanka wheat was relatively high, but the year was cool and damp and conditions seemed not to be as nearly optimum for this crop as during the hot, dry years. This is especially noticeable
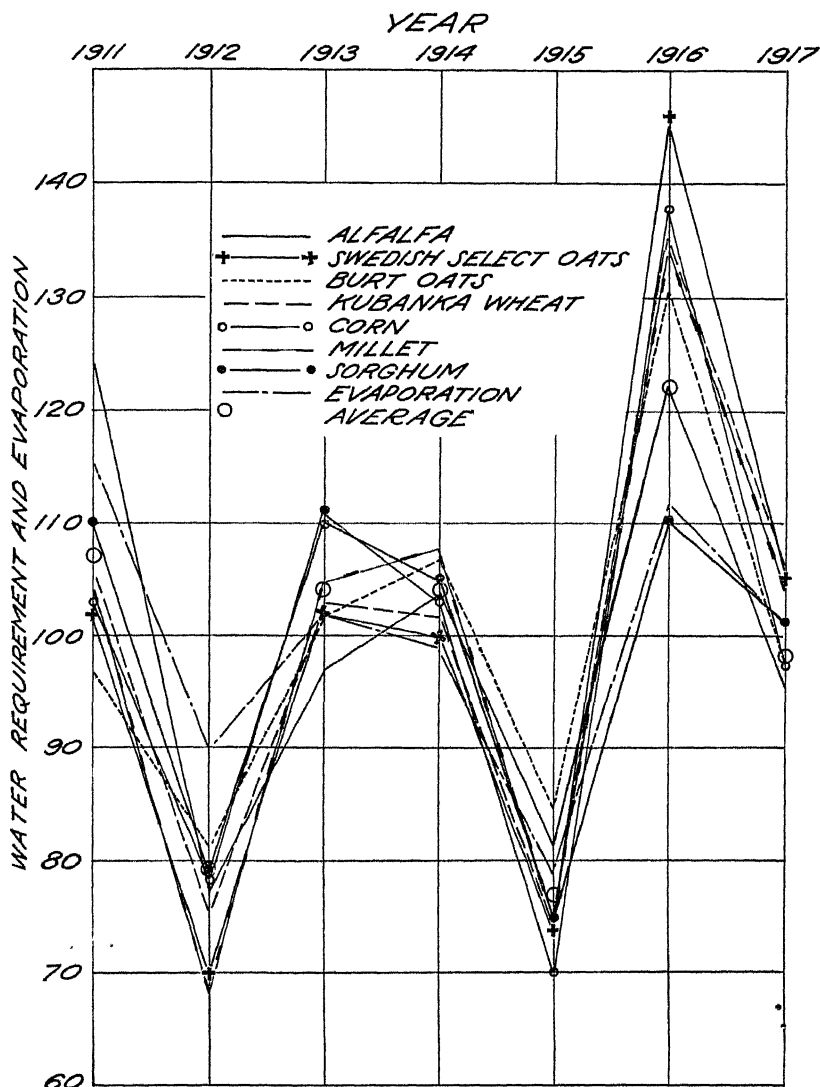
FIG. 7.—The water requirement of different crops and the evaporation expressed in percentages of the mean for the years 1911–1917

in the 1915 results, when Kubanka wheat had a higher relative water requirement value than any other crop. During 1916 the water requirement value of Kubanka wheat was as high as the mean water requirement value of all crops for the year. The alfalfa results are likely to be influenced by a factor that does not enter to the same degree in the

case of wheat, which has only a single crop, as compared with from two to four crops in the case of alfalfa. For instance, the water requirement of alfalfa, on the basis of season's growth, for a hot month of one year, when the crop is at its maximum growth, as compared with the water requirement of alfalfa during the same month of another year, when the crop has just been cut and the plants are small, may be quite different. In general terms, the results with alfalfa are in accordance with the mean of the eight crops grown. In 1911 the value was 17 points too high; in 1914, 1 point too high; and in 1915, 4 points too high, although the latter was an unusually cold and damp year. The water requirement of alfalfa for all other years was low, especially in 1917, 1916, 1913, and 1912, when the values were 5, 9, 7, and 2 points, respectively, below the mean of the eight crops grown in the experiment.

TABLE 30.—*Dry matter per pot expressed in grams and in index numbers of the mean value for each crop taken as 100, of all crops grown at Akron, Colo., for the periods 1911–1917, inclusive*

DRY MATTER PER POT (IN GRAMS)

Crop	1911	1912	1913	1914	1915	1916	1917	Average
Alfalfa, A. D. I. E–23	196.4	337.8	292.2	305.9	216.5	123.4	183.5	236.5
Oats, Swedish Select, C. I. 134	173.7	396.0	275.5	264.5	332.4	103.2	170.9	245.1
Oats, Burt, C. I. 293	143.1	342.0	248.4	250.8	232.8	85.5	153.8	208.1
Barley, Hannchen, C. I. 531	136.3	297.3	(a)	181.9	261.7	103.7	139.6	186.8
Wheat, Kubanka, C. I. 1440	94.2	274.1	280.4	305.7	237.7	91.7	153.1	205.3
Corn, Northwestern Dent	160.7	432.0	356.3	304.4	112.0	180.6	336.8	269.0
Millet, Kursk, S. P. I. 34771	202.0	242.7	211.3	301.2	256.0	171.0	229.2	230.5
Sorghum, Minnesota Amber, A. D. I. 341–13	307.6	487.2	605.5	457.1	203.2	192.8	276.2	361.4
Average	176.8	351.1	324.2	296.4	231.5	131.5	205.4	b 244.0

DRY MATTER PER POT EXPRESSED IN INDEX VALUES OF THE MEAN VALUE

Alfalfa, A. D. I. E–23	83	143	124	129	92	52	78	100
Oats, Swedish Select, C. I. 134	71	162	112	108	136	42	70	100
Oats, Burt, C. I. 293	69	164	119	121	112	41	74	100
Barley, Hannchen, C. I. 531	73	159		97	140	56	75	100
Wheat, Kubanka, C. I. 1440	46	134	137	149	116	45	75	100
Corn, Northwestern Dent	60	161	132	113	42	67	125	100
Millet, Kursk, S. P. I. 34771	88	105	92	131	111	74	99	100
Sorghum, Minnesota Amber, A. D. I. 341–13	85	135	168	126	56	53	76	100
Average	72	145	126	122	101	54	84	100

DRY MATTER PER POT (IN GRAMS)

				1914	1915	1916	1917	Average
Alfalfa, A. D. I E–23				305.9	216.5	123.4	183.5	207.3
Oats, Swedish Select, C. I. 134				264.5	332.4	103.2	170.9	217.8
Oats, Burt, C. I. 293				250.8	232.8	85.5	153.8	180.7
Barley, Hannchen, C. I. 531				181.9	261.7	103.7	139.6	171.7
Wheat, Kubanka, C. I. 1440				305.7	237.7	91.7	153.1	197.1
Corn, Northwestern Dent				304.4	112.0	180.6	336.8	233.4
Millet, Kursk, S. P. I. 34771				301.2	256.0	171.0	229.2	239.4
Sorghum, Minnesota Amber, A. D. I. 341–13				457.1	203.2	192.8	276.2	282.3
Cotton, Triumph				346.8	108.7	151.2	275.1	220.5
Cowpea, S. P. I. 29282				170.3	127.8	119.4	170.1	146.9
Pigweed				219.9	159.1	110.0	123.5	153.1
Grama grass				72.4	45.0	34.2	40.0	47.9
Rye, Vern, C. I. 73				195.2	202.9	83.7	121.2	150.8
Wheat, Galgalos, C. I. 2398				298.4	167.5	56.4	159.8	170.5
Sudan Grass, S. P. I. 25017				360.1	190.4	161.4	246.3	239.6
Average				268.7	190.2	117.9	185.3	190.5

 a No correction made for the absence of this value.   b True average.

TABLE 30.—*Dry matter per pot expressed in grams and in index numbers of the mean value for each crop taken as 100, of all crops grown at Akron, Colo., for the periods 1911–1917, inclusive—Continued.*

DRY MATTER PER POT EXPRESSED IN INDEX VALUES OF THE MEAN VALUE

Crop	1911	1912	1913	1914	1915	1916	1917	Average
Alfalfa, A. D. I. E–23				148	104	60	89	100
Oats, Swedish Select, C. I. 134				121	153	47	78	100
Oats, Burt, C. I. 293				139	129	47	85	100
Barley, Hannchen, C. I. 531				106	152	60	81	100
Wheat, Kubanka, C. I. 1440				155	121	47	78	100
Corn, Northwestern Dent				130	48	77	144	100
Millet, Kursk, S. P. I. 34771				126	107	71	96	100
Sorghum, Minnesota Amber, A. D. I. 341–13				162	72	68	98	100
Cotton, Triumph				157	49	69	125	100
Cowpea, S. P. I. 29282				116	87	81	116	100
Pigweed				144	104	72	81	100
Grama grass				151	94	71	84	100
Rye, Vern, C. I. 73				129	135	56	80	100
Wheat, Galgalos, C. I. 2398				175	98	33	94	100
Sudan Grass, S. P. I. 25017				151	79	67	103	100
Average				141	102	62	95	100

The two varieties of oats agree in water-requirement value fairly well, but diverge 11 points in 1916. Their values were remarkably low during the cool, damp years 1912 and 1915, but during the hot, dry year 1916 they were higher than for any other crops except corn. During the three years 1913, 1914, and 1917, when the evaporation rate was approximately the mean of the period, the water requirement of oats did not depart more than 6 points from its mean for the total period.

The water requirement of barley approximates the mean of all crops during the hot, dry years, but during the cool, damp years its water-requirement values were relatively high, 9 points in 1912 and 2 points in 1915. It resembles Kubanka wheat closely and is in contrast with oats varieties in its response to various years.

PROBABLE RANGE IN WATER REQUIREMENT OF CROPS

Tables 27 and 28 and Figures 6 and 7 indicate that crops react differently in different seasons. If in the seven-year period, 1911–1917, the average index value of the longest series of crops available is used, the highest water-requirement values, considering the results of experiments as a whole, were obtained in 1916, when the index value was 22 points above the average. This was an exceptionally dry year and the evaporation during July, when the crops were growing actively, was higher than for any month recorded at Akron during the period 1908–1925. (See Tables 31 and 32.) The lowest water-requirement values for the period were obtained in 1915, when the values dropped to 23 points below the average. The year was characterized by a low mean temperature, 50° F., and by a very low seasonal evaporation, lower than for any year during the period 1908 to 1924, inclusive. The year 1912 was almost as low and was characterized by low rates of evaporation, a low mean temperature, 59° F., and by reduced light intensity.[12] Of the remaining years,

[12] BRIGGS, L. J., and SHANTZ, H. L. RELATIVE WATER REQUIREMENT OF PLANTS. Jour. Agr. Research 3: 6. 1914.

1917 was almost the average for the series, while 1911, 1913, and 1914 were somewhat above the average. The variation in water requirement from year to year at Akron is very great, the range, during the period of the experiments here discussed, based on the mean, being about 45 points. The extreme range for a single crop was about 67 points for oats, and the lowest about 30 points for grama grass. The range in evaporation was about 37 points.

TABLE 31.—*Climatic conditions at Akron, Colo., from April to September, inclusive, for the years 1911–1917*

Month	Air temperature (°F)					Precipi-tation	Evapo-ration	Wind velocity per hour
	Average of—			Absolute				
	Mean	Maxi-mum	Mini-mum	Maxi-mum	Mini-mum			
						Inches	*Inches*	*Miles*
1911:								
April	47	62	31	77	16	2.63	5.81	9.1
May	58	73	43	91	28	1.15	7.32	9.7
June	70	87	53	98	43	1.48	9.75	7.4
July	70	86	55	95	40	1.34	9.77	7.1
August	69	86	54	99	41	1.30	8.94	8.1
September	64	80	50	95	39	2.40	7.18	7.0
1912:								
April	45	58	32	73	23	2.49	4.58	9.9
May	55	70	42	92	28	2.86	7.10	8.5
June	63	75	49	89	37	3.39	6.75	6.1
July	70	84	55	96	46	3.58	7.62	5.4
August	69	83	55	96	48	1.58	7.05	4.7
September	54	68	41	91	22	1.88	4.65	6.0
1913:								
April	47	63	33	84	10	2.19	4.41	8.1
May	57	72	44	91	31	1.41	5.84	6.9
June	67	82	53	97	37	1.35	8.18	8.1
July	72	88	55	103	43	1.85	9.26	6.1
August	75	91	59	98	53	1.14	9.30	5.6
September	58	70	45	92	27	2.08	6.01	6.4
1914:								
April	45	58	34	79	15	4.01	4.29	8.7
May	57	70	44	85	32	1.46	5.61	7.4
June	68	83	50	93	38	3.54	7.51	5.8
July	72	87	58	96	49	1.66	8.65	5.8
August	71	88	55	101	40	1.05	8.36	6.5
September	61	83	47	92	31	.23	7.44	6.9
1915:								
April	50	62	38	78	29	5.19	4.22	7.5
May	52	64	41	88	27	4.13	5.03	7.7
June	60	72	49	82	35	3.75	5.88	6.8
July	67	81	54	95	40	1.10	6.66	6.2
August	64	79	52	94	39	3.51	5.82	4.3
September	60	76	47	92	35	1.76	5.79	6.1
1916:								
April	44	59	30	84	11	1.50	6.21	8.1
May	55	70	41	92	27	2.24	7.81	9.3
June	64	80	49	99	33	2.09	7.98	7.4
July	75	93	60	98	54	1.77	11.12	7.3
August	69	83	56	95	45	2.82	7.22	5.4
September	61	77	45	91	30	.26	6.84	6.2
1917:								
April	44	58	29	88	15	.96	4.08	6.8
May	50	64	36	82	21	7.79	4.93	8.5
June	64	80	48	97	32	.56	8.42	7.4
July	73	90	57	99	47	1.52	10.19	5.5
August	67	82	52	97	42	1.78	8.46	5.8
September	64	79	48	95	39	2.19	6.62	6.0
Average or total:								
1911	63	79	48	99	16	10.30	48.80	8.1
1912	59	73	46	96	22	15.78	37.75	6.8
1913	63	78	48	103	10	10.05	43.06	6.9
1914	63	78	48	101	15	11.95	41.86	6.9
1915	59	72	47	95	27	19.44	33.40	6.5
1916	61	77	47	99	11	10.77	47.18	7.3
1917	60	76	45	90	15	14.80	42.70	6.7

TABLE 32.—*Precipitation and evaporation at Akron, Colo., from April to September, inclusive, for the years 1908–1925*

Year	April	May	June	July	August	September	Total
Precipitation (inches):							
1908	1. 70	3. 30	2. 37	2. 42	1. 47	0. 05	11. 31
1909	. 40	1. 87	3. 32	4 61	3. 77	2. 12	16 09
1910	3. 96	2. 06	1. 38	1. 47	3. 72	3. 81	16. 40
1911	2 63	1. 15	1. 48	1 34	1. 30	2. 40	10 30
1912	2. 49	2 86	3 39	3. 58	1 58	1. 88	15 78
1913	2. 19	1 44	1. 35	1. 85	1. 14	2. 08	10. 05
1914	4 01	1 46	3 54	1 66	1. 05	. 23	11. 95
1915	5 19	4. 13	3 75	1. 10	3 51	1 76	19. 44
1916	1 59	2. 24	2 09	1. 77	2. 82	. 26	10. 77
1917	. 96	7 79	. 56	1 52	1. 78	2 19	14. 80
1918	1. 20	1. 76	. 96	3. 10	7. 36	2. 43	16. 81
1919	1. 96	1. 59	2. 27	1. 79	. 44	2. 62	10. 67
1920	3. 28	2. 90	3. 97	4. 72	1. 45	1. 80	18 12
1921	2 77	. 47	1. 32	2. 88	. 92	. 79	9 15
1922	3. 96	3. 63	1. 43	3. 24	1. 24	. 06	13. 56
1923	1. 65	4 94	2. 17	3. 62	. 75	. 82	13. 95
1924	. 31	3. 26	. 35	1. 71	. 77	4. 04	10. 44
1925	2 24	1. 19	2 90	1. 08	1. 01	. 50	8. 92
Average 1908–1925	2. 36	2 67	2. 14	2. 41	2. 00	1. 60	13. 25
Average 1911–1917	2. 72	3. 01	2 31	1. 83	1. 88	1. 54	13. 29
Evaporation (inches):							
1908	4. 74	7. 71	8. 64	8. 47	7. 83	8. 55	45. 94
1909	4. 73	6. 83	7. 00	9 40	8. 54	5 86	42. 36
1910	6 39	5. 80	8. 72	9. 76	7. 14	5. 81	43. 62
1911	5 84	7. 32	9. 75	9. 77	8. 94	7. 18	48. 80
1912	4. 58	7. 10	6. 75	7. 62	7. 05	4. 65	37. 75
1913	4. 44	5. 84	8. 18	9 26	9. 30	6. 04	43. 06
1914	4. 29	5 61	7. 51	8. 65	8. 36	7. 44	41. 86
1915	4. 22	5. 03	5. 88	6. 66	5. 82	5 79	33. 40
1916	6. 21	7 81	7. 98	11. 12	7. 22	6. 84	47. 18
1917	4 08	4. 93	8. 42	10. 19	8. 46	6 62	42. 70
1918	4 10	6. 78	9. 33	9 25	7. 68	4. 28	41. 42
1919	4. 95	7. 38	8. 76	10. 28	9. 72	6 14	47. 23
1920	4. 79	6 84	6 96	8. 58	6. 92	6. 83	40. 92
1921	5. 51	6. 25	7. 77	10. 71	8. 59	7. 08	45. 91
1922	4. 28	6 79	8. 23	9. 20	8. 85	7. 23	44. 58
1923	5. 09	5. 48	7. 81	9. 06	7. 70	6. 29	41. 43
1924	4. 89	1 7. 65	8. 64	10. 54	10. 15	5. 82	47. 69
1925	5. 83	7. 32	8. 86	10. 27	8. 72	6. 30	47. 30
Average 1908–1925	4. 94	6. 58	8. 07	9 38	8. 17	6 38	43. 51
Average 1911–1917	4. 81	6. 23	7. 78	9. 04	7. 88	6. 37	42 11

¹ Interpolated mean, average from 1908–1923.

Northwestern Dent, a variety of corn grown in the northwest portion of the Great Plains was used in the experiments. During the hot, dry year 1916, its value was 7 points above the mean, but in 1915, a cool, damp year, its value was 6 points below. Northwestern Dent is most economical in its use of water, therefore, during cool, damp years, while its water requirement rises in hot, dry years.

Although it has a very low water requirement and is an excellent, dry-land crop, Kursk millet gave a relatively higher water requirement in hot, dry years than in cool, damp ones. Its requirement in 1916, a damp, cool year, was 3 points above the mean; while in 1912 and 1915 it was 10 and 3 points, respectively, below the mean.

Sorghum showed remarkable efficiency in the use of water during the hot, dry years. Its water requirement in 1916, for instance, was 21 points below the mean. During the cool, damp year 1912, on the other hand, it was 11 points above the mean.

The crops grown from 1911 to 1917, considered consecutively as to their range in water requirement, are as follows:

	Lowest index value	Highest index value	Range in points
Oats, Swedish Select_____	70	146	76
Corn, Northwestern Dent_____	71	138	67
Millet, Kursk_____	68	134	66
Oats, Burt_____	74	135	61
Barley, Hannchen_____	79	130	51
Wheat, Kubanka_____	81	131	50
Alfalfa, Grimm_____	76	124	48
Sorghum_____	75	110	35
Evaporation_____	79	116	37

Sorghum, alfalfa, wheat, and barley showed the low ranges in the order given. Swedish Select oats, corn, millet, and Burt oats showed high ranges. Only one crop, sorghum, was exceeded in range by evaporation.

The crops used in the experiments may be separated on the basis of the lowest relative water requirement recorded during cool, damp years and the highest water requirement recorded during hot, dry years, as follows:

	Departure from mean water requirement of crops in—		Difference[a]
	Cool, damp years	Hot, dry years	
Oats, Swedish Select_____	−8	+15	+23
Corn, Northwestern Dent_____	−6	+7	+13
Millet, Kursk_____	−10	+3	+13
Oats, Burt_____	−3	+4	+7
Barley, Hannchen_____	+2	−1	−3
Alfalfa, Grimm_____	−2	[b] −9	−7
Wheat, Kubanka_____	+3	0	−3
Sorghum_____	−2	−21	−19

[a] Those marked plus (+) are adapted to the cool, damp year, and those marked minus (−) to the hot, dry year.
[b] The outstanding value for 1911 is omitted.

This tabulation is practically a comparison of the 1912 or 1915 water-requirement results with those of 1916. The first four crops do much better during cool, damp years than the last four. Burt oats, which is low in water requirement, is relatively a much better hot-weather crop than Swedish Select oats. The last four crops do well during hot, dry years, the water requirement being at or below the average for the crops. The behavior of the crops, independent of actual values, is indicated by the index numbers in the last column. The crops which were most efficient during cool, damp years and least efficient during hot, dry years, are indicated at the top with the highest (+) values; and those which were relatively most efficient during hot, dry years and least efficient during cool, damp years, are indicated at the bottom by the highest (−) values.

The comparison of the results of water-requirement measurements in cool, damp years with the results in dry, hot years omits the outstanding water-requirement values of alfalfa in 1911. Had these been included, alfalfa would have had an index number of + 19, thus placing it first among the crops showing a low water requirement in cool, damp years.

The behavior of Kubanka wheat in 1916, when its index value was as low as the mean value for all crops, and in 1912 and 1915, when its index value was higher than the mean for all crops, indicates clearly that during cool years it is inefficient and places it among the crops which show the greatest relative efficiency in the use of water during the hot years; and a comparison of the results obtained in 1911, with those obtained in 1916, confirms this indication. (Compare figs. 8, 9, 10, and 11.) Alfalfa did not depart markedly from the mean of all crops except to fall 9 points below during 1916 and 17 points above in 1911. Since these years were both hot and dry, these variations are in opposite directions. Either or both may be regarded as outstanding. The fact that the result in 1911 is so great and is in direct opposition to that in 1916, may account to some extent for the slightly favorable performance during the other years. In any case, the 1911 result is probably outstanding, and if it were omitted, alfalfa would not be at the head of the crops with a low relative water requirement in cool weather, but rather among those relatively well adapted to hot, dry weather. It should be clearly borne in mind that this discussion does not concern itself with actual values but with relative performances of the same crop, during cool, damp and hot, dry years.

Cotton, which was included in the experiments for the period 1912–1917 ranged in water-requirement value from 81 to 120 points, or 39 points in all. It was inefficient during the cool years, its index value being 3 points above in 1915, and 9 points above in 1912, but very efficient during the hot, dry year 1916, when its value was 19 points below the mean of the crops grown that season. Cotton belongs with the warm season crops.

In the comparison of the water-requirement results for the crops tested during the period 1913 to 1917, cowpea, pigweed, and grama grass were added. They varied as follows: Cowpea, from 71 to 133, or 62 points in all; pigweed, from 76 to 113, or 37 points in all; grama grass, from 85 to 113, or 28 points in all.

A comparison of the water-requirement results for these three crops during cool, damp and hot, dry years on the basis of their relation to the mean water requirement shows the following departures from the mean:

	Cool, damp years	Hot, dry years	Difference
Cowpeas	−5	+11	+16
Pigweed	0	−9	−9
Grama grass	+15	−24	−39
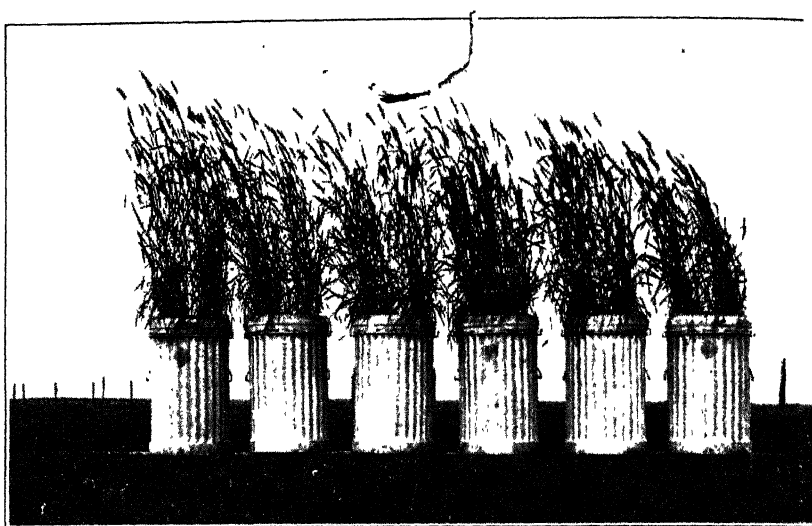
Fig. 8 —Kubanka wheat C I. 1140, grown in 1912, producing an average quantity of dry matter per pot of 274 grams, with a water requirement of 394. Based on the average for all years, the dry matter and water requirement index values for this crop were 134 and 81, respectively. Photographed at Akron, Colo., July 19, 1912

Fig. 9 —Kubanka wheat C. I. 1440, grown in 1913, producing an average quantity of dry matter per pot of 280 grams, with a water requirement of 496. Based on the average for all years, the dry matter and water requirement index values for this crop were 137 and 102, respectively. Photographed at Akron, Colo., July 8, 1913
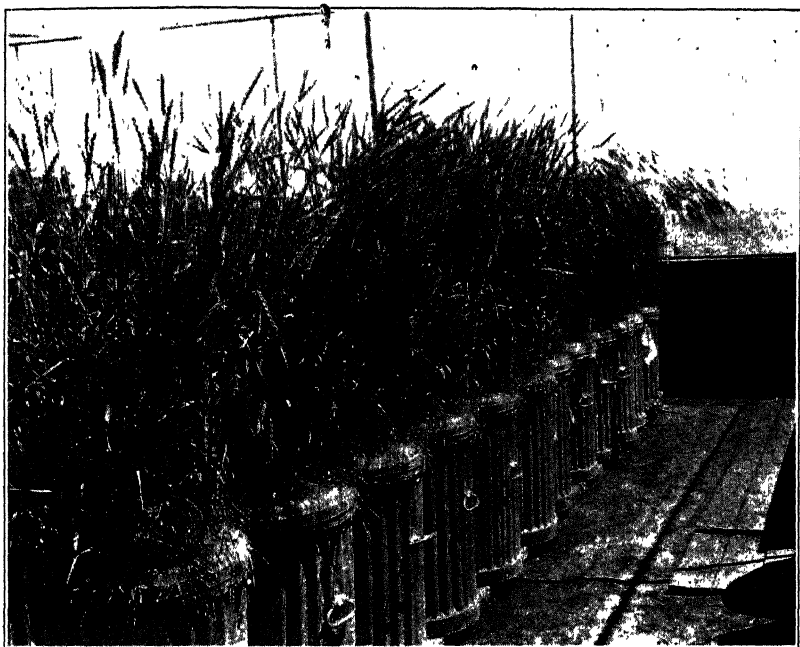
FIG. 10.—Kubanka wheat C. I. 1440, grown in 1914, producing an average quantity of dry matter per pot of 305 grams, with a water requirement of 518. Based on the average for all years, the dry matter and water requirement index values for this crop were 149 and 107, respectively. Photographed at Akron, Colo , August 11, 1913
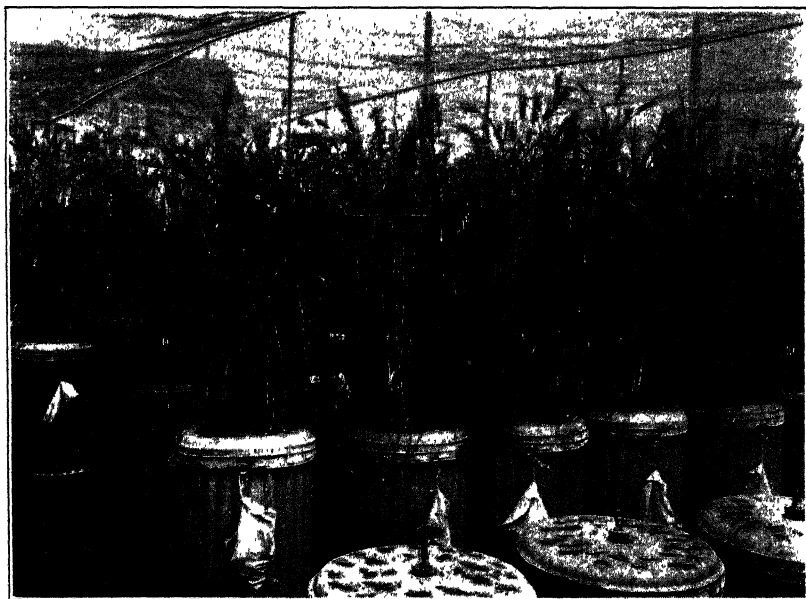
FIG. 11.—Kubanka wheat C. I. 1440, grown in 1915, producing an average quantity of dry matter per pot of 237 grams, with a water requirement of 405. Based on the average for all years, the dry matter and water requirement index values for this crop were 116 and 84, respectively. Photographed at Akron, Colo , August 20, 1915

53534—27——5

Cowpeas did not do as well during the hot, dry years as during the cool, damp year. Pigweed showed no advantage during the cool, damp year but was very efficient during the hot, dry year. Grama grass was inefficient during the cool, damp years but exceedingly efficient during the hot, dry year. Although grama grass does not produce, within its range, as efficiently as the cultivated crops, such as sorghum and millet, it is most efficient during hot, dry periods such as that experienced in 1916.

Rye, Galgalos wheat, and Sudan grass were added in the comparison of water-requirement results for the crops grown during the period 1914 to 1917. They varied as follows: Rye, from 75 to 127, or 52 points in all; Sudan grass, from 71 to 117, or 46 points in all; Galgalos wheat, from 84 to 113, or 29 points in all.

A comparison of the water-requirement results for these three crops during cool, damp seasons and hot, dry years on the basis of their relation to the mean water requirement shows the following departures from the mean:

	Cool, damp years	Hot, dry years	Difference
Rye	--2	+5	+7
Sudan grass	—6	—5	+1
Galgalos wheat	+7	—9	—16

Rye showed an efficient water requirement during the cool, damp year but an inefficient requirement during the hot, dry year. Sudan grass and Galgalos wheat were efficient during the hot, dry year but Sudan grass was efficient also during the cool, damp year, which reduced the value of the combined index and does not indicate a preference to either extreme. Galgalos wheat was very inefficient during the cool year. This was due in part at least to rust infection.

A comparison of the relative efficiency of the crops grown in 1915, a very favorable year, with those grown in 1916, a very unfavorable year, the former relatively damp and cool, the latter hot and dry, gives the following departures from the mean water requirement for the series:

	Departure from the mean		Difference
	1915	1916	
Oats, Swedish Select	—7	+15	+22
Corn, Northwestern Dent	—8	+13	+21
Cowpeas	--6	+10	+16
Oats, Burt	—6	+7	+13
Millet, Kursk	—7	+6	+13
Rye, Vern	—2	+5	+7
Barley, Hannchen	0	+5	+5
Sudan grass	—6	—5	+1
Mean of all crops	0	0	0
Wheat, Kubanka	+3	+3	0
Alfalfa, Grimm	+4	—1	—5
Pigweed	0	—7	—7
Sorghum, Minnesota Amber	0	—10	—10
Cotton, Triumph	+5	—8	—13
Wheat, Galgalos	+7	—9	—16
Grama grass	+17	—21	—38
Evaporation	+4	+8	—4

The low values in the first column represent water-requirement efficiency on the part of crops during the cool, damp year, and the high values represent inefficiency. The high values during the hot, dry year, 1916, represent inefficiency and the low values, efficiency. These values are combined in the last column in a linear scale. High values indicate crops which are efficient in the use of water during cool years and inefficient during hot years; while those of low value indicate crops which are inefficient during cool years and efficient during hot, dry years.

This table shows clearly that certain crops were more efficient in their use of water during the cool, damp year, 1916, and others during the dry, hot year, 1915. Corn, oats, cowpeas, and millet gave the lowest relative water requirement results during the damp, cool year; and grama grass, Galgalos wheat, cotton, and sorghim during the hot, dry year. The relative water requirement of Sudan grass and Kubanka wheat fall at or very near the mean water requirement for all crops. The position of Galgalos wheat is probably due to its susceptibility to rust, which increased the water-requirement value during 1915. The water-requirement values are all relative, but the rating and spread for the various crops are significant.

On the basis of the ratio of 1915 to 1916, the crops would fall in the following order, those having the lowest values being least adapted to a year like 1916 and best adapted to a year like 1915; and those having the highest ratios are best adapted to a year like 1916 and least adapted to a year like 1915: Wheat, C. I. 4131, 50; oats, Swedish Select, 51; corn, Northwestern Dent, 51; potato, Irish Cobbler, 52; alfalfa, Grimm E–23, 54; oats, Burt, 55; millet, Kursk, 55; rye, 59; Sudan grass, 61; barley, Hannchen, 61; wheat, Marquis, 61; wheat, Kubanka C. I. 4082, 62; flax, Jalaun, 62; wheat, Kubanka C. I. 1440, 64; alfalfa, A. D. I. E–23, 66; pigweed, 67; sorghum, Minnesota Amber, 69; Kashgar flax, 70; evaporation, 71; wheat, Pacific Bluestem, 71; cotton, 72; wheat, Galgalos, 74; grama grass, 93.

These values range from 13 below the average to 30 above, or 21 below evaporation to 22 above. The results are significant in that they show that the plants with high values which are at the bottom of the above list gave an unusually low relative water requirement during the hot, dry year 1916, and those with low values which are at the top of the list did best during the damp, cool year. These results confirm in a general way those based on Table 28 but are not as reliable, since only the results of two years are involved; while in the case of the results of crops grown for four years continuously the departures are taken from the mean. Either method affords a means of grading crops as to their relative efficiency in the use of water during extreme years.

EFFECT OF DIFFERENT SEASONS ON THE WATER REQUIREMENT AND THE PRODUCTION OF DRY MATTER

Although this study is concerned primarily with water requirement, the total amount of crops produced is important. It is possible for crops to utilize water very efficiently and yet to produce only a small amount of growth. This possibility is emphasized in the results here considered. In general there is a negative correlation between the

amount of total dry matter produced and either the water requirement or evaporation. The years of heavy growth were years of low evaporation and low water requirement. The negative correlation is not very close, due to the marked dissimilarity in the behavior of the different crops. The figures here presented are index values only, based on 100, and are taken from Tables 28 and 30. The temperature values are derived from an analysis of thermograph records for the years 1911 to 1916, inclusive. The total number of hours during which the temperature was above 80° F. for the period May to September was recorded for each year. The following are the index values of growth, water requirement, and evaporation for crops grown at Akron, Colo., as percentages of the mean fixed arbitrarily for the years 1911 to 1917 at 100.

	1911	1912	1913	1914	1915	1916	1917	Average
Growth	72	145	126	122	101	54	84	100
Water requirement	107	78	104	103	77	131	101	100
Evaporation	116	90	102	99	79	112	101	100
Hours with temperature above 80° F., April to September, inclusive	141	82	141	75	44	115		100

In general, when growth is low, evaporation and water requirement are both high, and vice versa. The correlation between growth and water requirement is -0.70 ± 0.13, and between growth and evaporation is -0.59 ± 0.17. The temperature record is startling in several respects. The temperature value for 1917, an average year, was omitted. The years 1911 and 1913 were exactly alike in hours with temperature above 80° F., but if only hours above 90° F. were considered, 1911 had only 65 per cent as many hours as 1913. On this basis, therefore, 1913 was a hotter year then 1911. Increased growth was recorded for Kubanka wheat, sorghum, and corn during 1913.

The water requirement of crops was slightly less in 1913 than in 1911, due largely to the excessively high value of the water requirement of alfalfa in 1911. The evidence indicates about equal water requirement for those two years but very unequal growth. Evaporation was higher in 1911 than in 1913 for the period April to September, inclusive, and for each month during the period with the exception of August. The data obtained seem to provide no adequate explanation for the greater amount of growth in 1913 as compared with 1911.

The 1912 results are more easily explained. Due to the low intensity of light in 1912 it was an exceptionally good year for the economic use of water and for the production of heavy crops. The index numbers show growth 45 points above the mean, evaporation 10 points below, hours above 80° F., 18 points below the average, and water requirement 22 points below average.

Both evaporation and water-requirement values were in 1914 near the mean, but temperature was 25 points below and growth 22 points above. It was a favorable year for Kubanka wheat and Kursk millet, but unfavorable for barley, Swedish Select oats, and corn.

Based on the mean of all the crops the 1915 crops made an average growth. The distribution of growth among the various crops, however, was very unequal. The water requirement, how-

ever, was 23 points and ẻvaporation 21 points below the mean. It was by far the coolest year encountered in this study, the temperature in hours above 80° F. being 56 points below the average. Apparently, the weather was too eool for certain crops.

In 1916 growth was 46 points below the mean or lower than for any other year. The water requirement was 31 points, evaporation 12 points, and temperature 15 points above the mean. It was a dry, hot year, and on the basis of results obtained in previous experiments was favorable to sorghum and alfalfa and unfavorable to oats, corn, and millet.

During 1917 crops showed a normal water requirement and evaporation, but a growth 16 points below the mean. On the basis of production, corn and millet were high while all other crops were low, Swedish Select oats being 14 points below the mean. On the basis of water requirement alfalfa, wheat, and corn were the most efficient and Burt oats the least efficient. On the basis of the larger series of crops, corn, cotton, cowpea, and Sudan grass were most productive, and Swedish Select oats, Kubanka wheat, rye, barley, and pigweed least productive. The most efficient in the use of water were cowpea, grama grass, and Kubanka wheat, and the least efficient Sudan grass, pigweed, and sorghum.

A comparison of the relative position of crops on the basis of total dry matter produced and of the water requirement for two extreme years, such as 1915 and 1916, is of interest. In the following tabulation the index values of the crops have been arranged in order, with the crops showing the lowest relative water requirement at the top of the list and those showing the highest relative water requirement at the bottom of the list. In the second column the departure above or below the mean in production of dry matter is recorded. The difference between the index values of the first and second columns is recorded in the third column:

Crop	Water requirement, 1915	Total dry matter, 1915	Difference, 1915
Corn	−8	−54	−46
Millet	−7	+5	+12
Oats, Swedish Select	−7	+51	+58
Cowpea	−6	−15	−9
Oats, Burt	−6	+27	+33
Sudan grass	−6	−23	−17
Rye	−2	+33	+35
Barley	0	+50	+50
Pigweed	0	+2	+2
Sorghum	0	−30	−30
Wheat, Kubanka	+3	+19	+16
Alfalfa	+4	+2	−2
Cotton	+5	−53	−58
Wheat, Galgalos	+7	−4	−11
Grama grass	+17	−8	−25

The figures in the comparison may be interpreted more readily by the use of the third column of figures. Minus values in the first column show low water requirement, while plus values in the second column show high dry-matter production. On the basis of water requirement only, the most efficient crops stand at the head of the

list and show the greatest minus departures, but on the basis of production those showing the greatest plus departures are the most efficient. These may be combined as in the third column. Crops in this column having the greatest plus values are those which are either efficient in the production of dry matter and economical in the use of water or which show both these characteristics. Swedish Select oats, with an index value of +58, and barley, rye, Burt oats, Kubanka wheat, and millet, with index values of +50, +35, +33, +16, and +12, respectively, gave the best performance records during this damp, cool year. The crops which made poor showings during the year are: Cotton with an index value of −58, and corn, sorghum, grama grass, Sudan grass, Galgalos wheat, and cowpea with index values of −46, −30, −25, −17, −11, and −9, respectively.

While the data here presented are not as complete as they might be, they indicate rather clearly that plants are delicately adjusted to different optimum conditions. The water-requirement and growth data for 1915 indicate that corn exhibits a high degree of efficiency in the use of water but that this efficiency is accompanied by a greatly reduced growth. Apparently the season was too cool for a good crop of corn, but the cool and damp weather cut down the water loss, which accounted for the low water requirement. The same was true also of cowpea and Sudan grass. Sorghum showed an efficiency in the use of water equal to the average efficiency of all crops, but produced a very small crop, while cotton was less efficient in the use of water and produced relatively less than any other crop. Although it did not fall so far below the mean of all the crops in production of dry matter, grama grass required for its growth a relatively great amount of water. All of these crops responded unfavorably to the cool season. Several other crops, however, responded favorably to the season. Swedish Select oats produced 51 points more dry matter than the mean production for all plants, the water requirement to produce which was 7 points below the average for all crops. Burt oats was almost equally efficient in the use of water, but did not produce as much dry matter, while rye, only 2 points more efficient in the use of water than the average for all crops, was 33 points more productive in dry matter. Although barley was not above the average in efficiency, it was 50 points above the average in production. Kubanka wheat was 3 points less efficient but 19 points more productive than the average for all crops.

In the results of the 1916 experiments the relative positions of the crops mentioned above are somewhat different. There is a general tendency, however, for those crops which stood high in efficiency in use of water in 1915 to stand low in 1916, and vice versa.

While the hot, dry season of 1916 caused all plants to have a high water requirement, it did not by any means affect them equally. Oats, corn, and cowpea used relatively a great amount of water, while grama grass, sorghum, Galgalos wheat, cotton, and pigweed were relatively efficient. On the basis of total production, however, neither the same relative position nor the reverse position is maintained. Cowpea, corn, pigweed, grama grass, millet, and cotton were relatively most productive, while Galgalos wheat, Kubanka wheat, and the oats varieties were least productive.

On the basis of dry matter production, cowpea, which produced a quantity 19 points above the average for all crops, ranked first in the 1916 tests. This dry matter, however, was produced at a water requirement 10 points above the average for all crops. Like cowpea, corn also gave a relatively high dry matter yield, 15 points above the average for all crops but at a water requirement 13 points above the average for all crops. Millet made a similar record, producing a quantity of dry matter 9 points above at a water requirement 6 points above the average for all crops. The exceptional production made by these three crops was obtained only with the abundant use of water. The high temperature not only favored their growth but also increased their transpiration.

Crop	Water require-ment, 1916	Total dry matter, 1916	Difference, 1916
Grama grass_____	−21	+9	+30
Sorghum_____	−10	+6	+16
Wheat, Galgalos_____	−9	−29	−20
Cotton_____	−8	+7	+15
Pigweed_____	−7	+10	+17
Sudan grass_____	−5	+5	+10
Alfalfa_____	−1	−2	−1
Wheat, Kubanka_____	+3	−15	−18
Barley_____	+5	−2	−7
Rye_____	+5	−6	−11
Millet_____	+6	+9	+3
Oats, Burt_____	+7	−15	−22
Cowpea_____	+10	+19	+9
Corn_____	+13	+15	+2
Oats, Swedish Select_____	+15	−15	−30

Another group of plants tested gave a good dry matter production with an economical use of water. In this group, grama grass made the best record, producing 9 points more dry matter than the average with a water requirement 21 points less than the average. Pigweed produced a quantity of dry matter 10 points above at a water requirement 7 points less than the average for all crops; while sorghum produced a quantity of dry matter 6 points above with a water requirement 10 points less than the average; and cotton a quantity of dry matter 7 points above at a water requirement 8 points less than the average. Here is a group of plants that not only produced more dry matter than the average for all crops during this hot dry year but did so at an increased relative efficiency in the use of water.

The high temperature was very unfavorable to some of the crops, however. Swedish Select oats, for instance, produced a quantity of dry matter 15 points less at a water requirement 15 points above the average for all crops. Burt oats gave a similar result, producing a quantity of dry matter 15 points less at a water requirement 7 points higher than the average for all crops. Kubanka wheat also fell 15 points below the average in dry matter production and was 3 points high in water requirement. Rye with a dry matter production 6 points below and a water requirement 5 points above the average for all crops; and barley with a growth 2 points below and a water requirement 5 points above the average for all crops, complete the list of plants showing poor dry matter production and high-water requirement during 1916. Galgalos wheat which fell 29 points below on dry matter production and was 9 points below

the average for all crops in water requirement, should probably have been included in the above series. The water requirement value is influenced by the fact that the values during damp years have been considerably raised by rust. It is probable, therefore, that this value should fall nearer to that of Kubanka wheat than is indicated by the records.

On the basis of efficiency in the use of water, grama grass stands at the head and Swedish Select oats at the bottom of the list. This is almost exactly the reverse of the results obtained in 1915. Of the plants showing the best combined result, that is, high productivity and efficiency in the use of water, grama grass, with an index value of +30, stands first, followed by pigweed, sorghum, cotton, Sudan grass, and cowpea, with index values of +17, +16, +15, +10, and +9 points, respectively. The poorest showing was made by Swedish Select oats, with an index value of −30, and Burt oats, Galgalos wheat, Kubanka wheat, rye, and barley, with index values of −22, −20, −18, −11, and −7 points, respectively.

A comparison of the figures representing the combined departures of different crops during 1915 and 1916 from the mean water requirement for all crops, is shown in the tabulation on page 1158.

It is clear from this comparison that the crops that were efficient in 1915 were, as a rule, inefficient in 1916, and that those most efficient in 1916 were inefficient in 1915. The highest minus values in the third column below are for the crops which were efficient in the use of water in 1915 and inefficient in 1916. Those at the bottom of the column with the highest plus values were efficient during 1916 and inefficient during 1915. It is somewhat surprising to find corn, cowpea, and millet so efficient in the use of water during the cool year 1915 and so inefficient during 1916. The position of Galgalos wheat is due, at least in part, to the fact that the crop rusted during the cool, damp year, 1915. This affected the value not only for this year but also the values for all other years as well.

If the figures representing combined departures of crops during 1915 and 1916 from the mean dry matter measurement are compared the order of the crops differs considerably from the order they present when compared on the basis of water requirement departures, as shown by the following tabulation:

Crop	Dry matter produced in—		Difference
	1915	1916	
Oats, Swedish Select	+51	−15	+66
Barley	+50	−2	+52
Oats, Burt	+27	−15	+42
Rye	+33	−6	+39
Wheat, Kubanka	+19	−15	+34
Wheat, Galgalos	−4	−29	+25
Alfalfa	+2	−2	+4
Millet	+5	+9	−4
Pigweed	+2	+10	−8
Grama grass	−8	+9	−17
Sudan grass	−23	+5	−28
Cowpea	−15	+19	−34
Sorghum	−30	+6	−36
Cotton	−53	+7	−60
Corn	−54	+15	−69

On the basis of dry matter production, crops showing high plus values were favored by 1915 and held back by conditions in 1916. The crops showing high minus values were favored by the hot year, 1916, and were greatly retarded in growth by the cooler year, 1915. The hot season crops on the basis of dry matter production are corn, cotton, sorghum, cowpea, Sudan grass, and grama grass, while the cool-weather crops are oats, barley, rye, and wheat.

If the results of the experiments based on dry matter and those based on water requirement are compared it will be seen that the crops are not similarly arranged. In other words, it does not follow that a crop efficient in the use of water will produce a big yield or that a high yield is produced with efficient use of water.

The crops which responded favorably to the cool season and unfavorably to the hot season in both water requirement and dry matter production were: Swedish Select oats, barley, Burt oats, and rye. Those that responded favorably to the hot season and unfavorably to the cool season were cotton, grama grass, and sorghum. The other crops were either inconsistent in their response or did not show any decidedly better performance either year. This may indicate a wider range of adjustment to weather conditions or an adaptation to mean conditions. Corn, which was efficient in the use of water was inefficient in the production of dry matter in 1915. In 1916, however, it was inefficient in the use of water but efficient in the production of dry matter. Cowpea responded in a similar way in the experiments for 1915 and 1916. Kubanka wheat showed no difference in water requirement but produced a heavy crop in 1915 and a light crop in 1916. These differences are emphasized by combining the plus and minus water requirement values and the plus and minus dry matter production values as follows:

Crop	Difference of departure from mean in water requirement in 1915 and 1916	Difference in departure from mean in total dry matter produced in 1915 and 1916	Combined results
Oats, Swedish Select	+22	−66	+88
Barley	+5	−52	+57
Oats, Burt	+13	−42	+55
Rye	+7	−39	+46
Wheat, Kubanka	0	−34	+34
Millet	+13	+4	+9
Wheat, Galgalos	−16	−25	+9
Alfalfa	−5	−4	−1
Pigweed	−7	+8	−15
Cowpea	+16	+34	−18
Sudan grass	+1	+28	−27
Sorghum	−10	+36	−46
Corn	+21	+69	−48
Grama grass	−38	+17	−55
Cotton	−13	+60	−73

High values such as shown by Swedish Select oats indicate the ability of a crop to produce a good yield and at the same time be efficient in the use of water during a season such as 1915, and to pro-

duce a poor yield and consume a relatively great amount of water during a season such as 1916. (Compare figs. 12, 13, 14, 15, and 16.) The low values such as shown for cotton indicate good yield and efficient use of water during hot seasons like 1916 and poor yield and inefficient use of water during cool seasons like 1915. In other words, crops with high values are favored by cool, damp seasons and those with low values by hot, dry seasons, measured in terms of Akron climate.

It is not necessary to repeat here the values or the order shown in the table. The cool season crops as shown by these figures are oats, barley, rye, wheat, and millet; the hot season crops, cotton, grama grass, corn, sorghum, sudan grass, cowpea, and pigweed. Alfalfa stands at the mean of this series of crops.

It is evident from the data here presented that crops when arranged in increasing order as to water requirement in one location or during
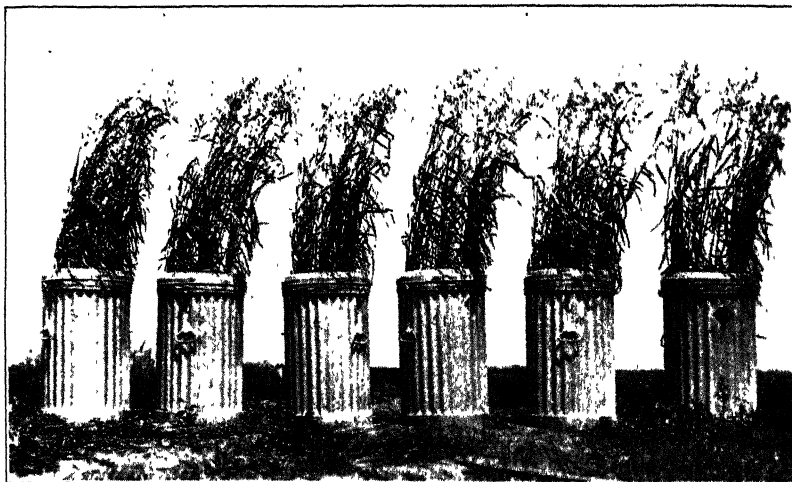
Fig. 12.—Swedish Select oats, grown in 1911, producing an average quantity of dry matter per pot of 174 grams, with a water requirement of 615. Based on the average for all years, the dry matter and water requirement index values for this crop were 71 and 102, respectfully. Photographed at Akron, Colo., July 22, 1911

one season can not be expected to maintain the same order when conditions are changed; that each crop is nicely adjusted to optimum conditions and that the optimum for growth may not necessarily be the optimum for economic use of water. Probably the most economical use of water would be recorded where plants were grown with conditions of temperature somewhat too low and humidity somewhat too high, but any departure from this optimum would increase the water requirement; and if the temperature is too high and the air too dry the water requirement would rapidly increase as compared with growth. If only transpiration were considered the conditions would be even more extreme. The data also shows the danger of phytometer work, which involves the assumption that results obtained with one species of plant can be rigidly applied to another.

₱HYTOMETRY

The great difference in behavior of crops here recorded indicates to some extent the danger of placing too much reliance upon, or making too broad an application of, phytometric records. For these two years the results of phytometer measurements might differ by almost 60 points. While the plant measures the environment, it measures it only in terms of its own requirements and the various plants of a population will differ much more from each other than they would from such a physical approximation as evaporation. If the evaporation is embodied as are the individual plants, the value falls surprisingly near the mean for all the plants here grown.

Phytometry is now receiving considerable attention by experimental ecologists. It is clear, from the behavior of this extensive
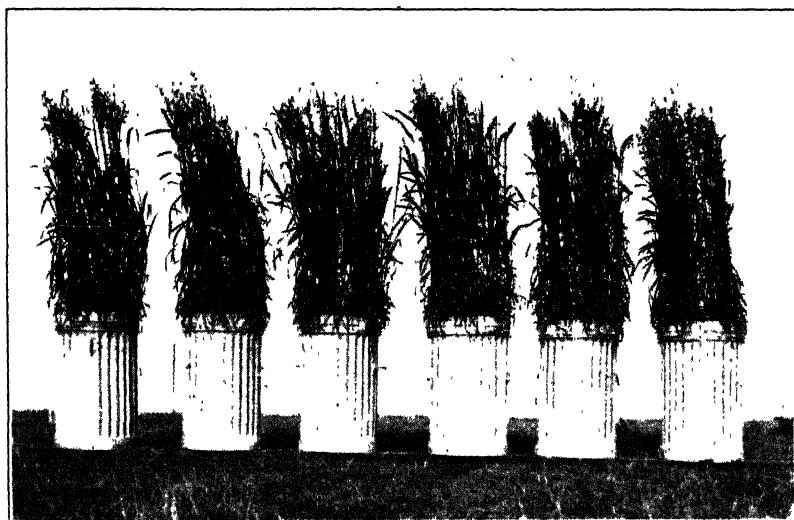
Fig. 13.—Swedish Select oats, grown in 1912, producing an average quantity of dry matter per pot of 396 grams, with a water requirement of 423. Based on the average for all years, the dry matter and water requirement index values for this crop were 162 and 70, respectively. Photographed at Akron, Colo., July 25, 1912

series of plants that they respond very differently to variations in weather conditions. In all probability no plant is a correct phytometer for any other plant. On the whole, plants are much more responsive to dryness of the air and the conditions that favor water loss than is a water surface. The evaporation range for the period 1911 to 1917 (Table 28), varied from 79 points for 1915 to 116 points for 1911, or 37 points, while the water requirement for eight representative crops ranges from 77 points for 1915 to 131 points for 1916, or 54 points. For the hot weather crop, sorghum, the water requirement varied from 75 points for 1915 to 110 points for 1911 and 1917, a range of 35 points. For the cool weather crop, Swedish Select oats, the water requirement varied from 70 points for 1912 to 146 points for 1916, a range of 76 points, more than twice as much as sorghum. Grama grass gave high water requirement values during the years 1913 and 1914, when conditions were not extreme; very high values
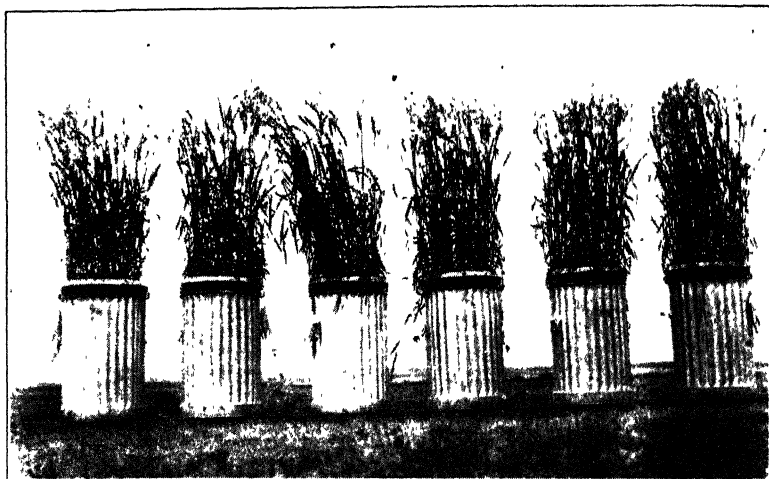
FIG 14 —Swedish Select oats, grown in 1913, producing an average quantity of dry matter per pot of 276 grams, with a water requirement of 617. Based on the average for all years, the dry matter and water requirement index values for this crop were 112 and 104, respectively. Photographed at Akron, Colo., July 19, 1913

FIG. 15.—Swedish Select oats, grown in 1914, producing an average quantity of dry matter per pot of 265 grams, with a water requirement of 599. Based on the average for all years, the dry matter and water requirement index values for this crop were 108 and 100, respectively. Photographed at Akron, Colo., August 1, 1914

during 1915, a year of low evaporation; and unusually low values during 1916, a year of high evaporation.   In other words, sorghum and grama grass are unusually efficient during hot dry years.   Oats is unusually efficient during cool, damp years.   To choose any one of these plants as a general measure of climatic conditions would introduce all the errors due to the adaptability of the plant to a narrow range of conditions.   Phytometry provides a method of determining the relative adaptability of a plant to special conditions through the measure of its efficiency in the use of water.

CORRELATION OF WATER REQUIREMENT WITH EVAPORATION

The figures here presented do not adequately represent the correlation of evaporation and water requirement, since no account is

FIG. 16.—Swedish Select oats, grown in 1916, producing an average quantity of dry matter per pot of 103 grams, with a water requirement of 876.   Based on the average for all years, the dry matter and water requirement index values for this crop were 42 and 146, respectively.   Photographed at Akron, Colo., August 2, 1916

taken of the period of growth and relative size of the crop during each period.   The evaporation data are for the six months' period, April to September, while most of the growth of crop plants is made during the latter part of June, July, and the early part of August. A very close agreement between the water requirement and evaporation would, therefore, not be expected.   It is necessary here only to point out that the variation of evaporation during the different years is not as great as that of transpiration, and a tendency on the part of the plant is to give a water requirement much higher during extreme years and much lower during the favorable years than would be expected from the evaporation data.   In other words, there is a

wider range in the water requirement of plants in proportion to season than there is in evaporation.

The population is so small, there being only seven observations in evaporation, that the correlation is not as significant as might be desired. The correlation of evaporation and transpiration for the different periods is as follows: 1911 to 1917, 0.76 ± 0.04; 1912 to 1917, 0.87 ± 0.02; 1913 to 1917, 0.82 ± 0.03; 1914 to 1917, 0.87 ± 0.02. This correlation, although only an approximation, indicates a very high degree of dependence of water requirement on evaporation.

The following are the correlations of each crop with the seasonal evaporation: Grimm alfalfa, 0.74 ± 0.11; Swedish Select oats, 0.76 ± 0.11; Burt oats, 0.79 ± 0.09; Hannchen barley, 0.86 ± 0.07; Kubanka wheat, 0.67 ± 0.16; Northwestern Dent corn, 0.76 ± 0.11; Kursk millet, 0.74 ± 0.11; Minnesota Amber sorghum, 0.90 ± 0.05.

It is evident from the consideration of the individual crops and their response to different types of seasons, that no close correlation would be likely between evaporation and each of the single crops. It is apparent, however, that water requirement depends to a large extent on evaporation. This correlation is shown also in the graphs in Figures 6 and 7.

RELATIVE WATER REQUIREMENT AT AKRON, COLO.

There are two chief causes of variation in the water requirement measurements presented in this study: (1) The kind of crop, and (2) the character of the growing season. Other causes were largely eliminated by the method of experimentation. In order to bring out more clearly the effect of season or year on the water requirement, the results have been arranged (Table 27) in such a way as to give the greatest number of crops used in the determinations during the period of experimentation.

In order to reduce the measurements made during different years to a comparable scale in which the relative water requirements of the different crops can be expressed, it is necessary to weight the values for each year. This has been done in accordance with the values shown in Table 27 for the respective years. Index values derived from the actual values shown in Table 27 are given in Table 28. These index values show that the plants do not respond proportionately season to season. Therefore it is impossible to weight these values without considerable error and the weighted values are probably not what they would have been had the crops been grown for the whole period of experimentation, nor is the mean of these values equal to 100; but the evident mathematical discrepancy is more than offset by the use of the longer crop series where available. These are therefore relatively close approximations and the errors due to crop variation are usually under 10 per cent and never exceed 21 per cent during any one year. With the data available this is as close an approximation as seems justified. It seemed best to the writers to present the material in this form; otherwise the reader would be left to make his own scale. The values of the results for each crop have been raised or lowered in proportion to the following scale of index values: 1911, 107; 1912, 80; 1913, 104; 1914, 104; 1915, 77; 1916, 122; 1917, 97.

In other words, the results of the year 1911 have been lowered by 7 points and those of 1912 raised by 20 points. By this method it has been possible to give in the final table, a weighted column in which the relative values of the different crops are presented. (See Tables 33 and 34.) These are estimates based on actual measurements for one or more years, and in the case of some crops represent approximately the actual average of the seasons where the crop has been grown for a period of seven years. The slight discrepancy between the actual averages and the weighted mean is due to the fact that more crops were included in the values for the later years than were included in 1911. Had only the crops which were included in the 1911 value been considered, there would be no discrepancy between the weighted and actual mean. The method here employed, it is believed, gives truer values when applied to a wide range of plants.

TABLE 33.—*Actual water requirement, based on total dry matter, and the weighted mean for each variety, genus, and crop or group, of all plants grown at Akron, Colo., during the years 1911–1917, inclusive*

Plant	1911	1912	1913	1914	1915	1916	1917	Number of years for which record was made	Species or variety	Genus	Crop or group
Proso (Panicum miliaceum):											
Black Voronezh, C. I. 15		206±1						1	258±1		
Tambov, S. D. 366, Akron 366-1-0		209±1						1	260±1		
Black Voronezh, S. D. 331		226±7						1	283±9	267±5	267±5
Millet. (Chaetochloa italica):											
Kursk, S. P. I. 30029		173±10						1	216±13		
Kursk, S. P. I. 22420	287±2	187±2	286±4	205±2	202±1	367±4	284±5	6	274±3		
Kursk, S. P. I. 34771		248±7						2	278±12		
German, S. P. I. 26845	263±15							1	304±5		
Siberian, A. D. I. 3-4		294±6						1	308±8	255±8	285±8
Turkestan, S. P. I. 20694											
Sorghum (Andropogon sorghum):											
Sorgo—											
Minnesota Amber, A. D. I. 341-13		239±2	298±2	284±3	203±3	296±4	272±5	6	274±3		
Dakota Amber, A. D. I. 341-10-A		237±4	296±1	296±1				1	285±1		
Red Amber, S. P. I. 17543	298±4							3	287±3		252±3
Grain sorghum—											
Brown Kaoliang, S. P. I. 24963	301±3	223±1						2	282±2		
Blackhull Kafir, S. P. I. 24975	278±5	250±5						2	292±6		
White Durra, S. P. I. 24997	321±2	255±5						2	312±3		
Milo, S. P. I. 24960		249±3						2	311±4		
Dwarf Milo, S. P. I. 24970	333±3	273±4						2	324±4		304±4
Grass sorghum—											
Sudan grass, S. P. I. 25017 (var. Aethiopicus)		359±2		394±4	260±3	426±3	378±3	5	350±3	365±3	380±3
Corn (Zea mays):											
Esperanza	319±5	239±3						2	299±5		
Tom Thumb								1	303±5		
Algeria								1	317±4		
Indian Flint			342±5					1	329±5		
Budapest		255±7	350±8	345±3				1	332±3		
Hopi				365±7				2	347±9		
Pima				368±9				1	351±7		
Joaquin				372±8				1	351±9		
German C24-2				398±6				1	358±3		
Northwestern Dent	368±10	280±10	399±12		253±7	465±13	346±3	1	361±10		
Laguna		255±6						1	369±8		
German C24-1								1	370±5		
China White		316±7	415±4					3	375±7		
Iowa Silvermine	420±3	302±7		344±7				2	386±7		
Bloody Butcher			405±7					1	389±7		349±7

Name							n		
Hybrids—									
China White×Esperanza						313±3	1		
China White×Hopi						332±3	1		
Algeria×China						334±5	1		
Joaquin×Budapest						351±5	1		
German C24-1×2						358±1	1		
China White×Laguna						361±5	1		
Budapest×Pima						373±5	1	349±6	350±5
Joaquin×Pima						374±9	1		
Teosinte (Euchlaena Mexicana):									
Durango	250±2		390±11			375±11	1	375±11	375±11
Hybrids—									
China White×Teosinte	289±4		376±4			362±4	1	362±4	362±4
Sugarbeet (Beta vulgaris):									
Morrison grown, Kleinwanzleben	377±8	321±8	347±5			377±9	2	377±9	377±9
Tumbleweed (Amaranthus graecizans)	275±7		365±5	229±3	340±13	260±6	6	283±6	
Pigweed (Amaranthus retroflexus)	336±4	320±7	372±1	306±1	307±6	305±6	2	314±5	
Russian thistle (Salsola pestifer)	336±5					314±5	1		
Lamb's quarters (Chenopodium album)	801±41		705±50		666±27	658±32	2	658±32	443±26
Polygonum (Polygonum aviculare)						678±48	1	678±48	
Barley (Hordeum vulgare):									
Beardless, C. I. 716	544±9	408±8		390±2		506±9	2	506±9	
Beldi, C. I. 190	543±2	416±4		387±3		514±4	2	514±4	
Hannchen, C. I. 531 (Hordeum distichon)	527±8	443±3	501±5	404±11	664±9	523±8	6	622±?	518±6
Nepal, C. I. 595	542±3	439±1				528±2	2	540±12	540±12
Buckwheat (Fagopyrum vulgare)	578±13					540±12	1		
Wheat:									
Emmer, C. I. 2951 (Triticum dicoccum)	534±14	428±3	458±10	390±2		517±10	2	517±10	517±10
Durum (Triticum durum)—									
Beloturka, C. I. 3705, S. P. I. 35480				387±3		483±6	2	483±6	
Beloturka, C. I. 3705 (old seed)	468±8	394±7	518±6	405±3	636±14	491±7	1		
Kubanka, C. I. 1440			496±10	396±6		496±10	7		
Jumillo, C. I. 1736			507±8	364±6	719±15		2		
C. I. 4131, S. P. I. 3715 (from Siberia)				357±4	717±30		2		
C. I. 4131 (old seed)					712±10		1		
C. I. 4131 (first generation)						531±13	1	531±13	
C. I. 4131 (second generation)							3		
C. I. 4082, S. P. I. 36388 (from Peru)			538±5	431±3	666±24	548±12	2	548±12	
C. I. 4082 (old seed)				413±6	710±9		1		
C. I. 4082 (first generation)					683±18		1		
C. I. 4082 (second generation)					696±30		1		
Kubanka, C. I. 2094						570±25	1	570±25	
Common (Triticum aestivum)—									
Turkey, C. I. 1571	506±3	364±6			526±63	455±8	1		
Kharkof, C. I. 1583		365±6			510±9	456±8	1		
Ghirka, C. I. 1517		457±3				529±38	3		
Power, C. I. 3697						530±9	1		
Marvel Bluestem, C. I. 3082	531±5	451±4	510±3	452±6	524±4	534±5	2	542±14	
Preston, C. I. 3328						539±7	2		
Glyndon, C. I. 2873						540±4	1		

TABLE 33.—*Actual water requirement, based on total dry matter, and the weighted mean for each variety, genus, and crop or group, of all plants grown at Akron, Colo., during the years 1911–1917, inclusive—Continued*

Plant	1911	1912	1913	1914	1915	1916	1917	Number of years for which record was made	Species or variety	Genus	Crop or group
Wheat—Continued.											
Common—Continued.											
Marquis, C. I. 3641 (old seed)				498±5	424±3	680±12		2			
Marquis, C. I. 3641 (first generation)					412±3	726±5		2			
Marquis, C. I. 3641 (second generation)						713±19		1	550±8		
Prelude, C. I. 4323						536±28		1	553±29		
Galgalos, C. I. 2398	496±4			624±5	481±4	652±8	543±6	5	557±5		
Pioneer							545	1	562		
C. I. 4090, S. P. I. 36502, Turkestan				624±5	469±4			2			
C. I. 4090 (old seed)				567±6	461±5			1	584±6		
Haynes Bluestem, Minn. 169, C. I. 2874				679±3	491±4	743±11	572±7	2	600±8		
Pacific Bluestem, C. I. 4067				639±2	501±4	690±7		3	619±5		
C. I. 4087, S. P. I. 36499					505±3			2	640±4		
C. I. 4087 (old seed)				689±8				1	663±8		
C. I. 4103				[a]916±15				1	[a]881±14		
C. I. 4127								1			557±13
Hybrids—											
Kubanka×Haynes Bluestem				574±2	417±2	636±21		1	521±17		
Jumillo×Preston			657±11					2	547±3	545±14	534±12
Cotton:											
Triumph (Gossypium hirsutum)	448±11	488±14		574±9	443±8	612±9	522±4	6	568±10	568±10	568±10
Potato:											
Irish Cobbler (Solanum tuberosum)			659±15		329±4	630±17		4	499±11		
McCormick (Solanum tuberosum)			717±11			744±28		2	630±18	[a]543±13	575±15
Oats:											
Canadian C. I. 444 (Avena sativa)	598±14	399±6						2	529±11		
Sixty-Day, C. I. 165 (Avena sativa)	605±5	491±13	617±9	599±2	448±10	876±21	635±5	7	594±12		
Swedish Select, C. I. 134 (Avena sativa)	615±7	423±5		615±6	445±5	809±5	636±5	7	604±10		
Burt, C. I. 293 (Avena sativa)	639±7	449±3	617±5					7	606±5	553±10	583±10
Native weeds:											
Purslane (Portulaca oleracea)			292±11					1	281±11	281±11	
Cocklebur (Xanthium commune)			432±13					1	415±13	415±13	
Nightshade (Solanum triflorum)								1	487±13		
Buffalo Bur (Solanum rostratum)				506±14				1	536±7		
Gumweed (Grindelia squarrosa)		468±18		557±7				1	585±23	[b]543±13	
Sunflower, annual (Helianthus annuus)			705±8					2	577±8		
Sunflower, narrow-leaved from sand-hills (Helianthus petiolaris)			570±11			579±10		1	548±11	585±29	

Plant								n			
Sunflower, narrow-leaved from heavy soil (Helianthus petiolaris)	765±24	474±14	774±20					1	744±10	623±13	
Mountain sage (Artemisia frigida)								2	654±20	654±20	
Verbena (Verbena bracteosa)								1	702±19	702±19	
Fetid marigold (Boebera papposa)			881±26	730±20				1	847±25	847±25	580±16
Crucifers:											
Cabbage, Early Jersey Wakefield (Brassica oleracea capitata)			539±7					1	518±7		
Turnip, purple top (Brassica rapa)			639±31					1	614±30		
Rape (Brassica napus)	a441±12		743±7					1	714±7	615±18	615±18
Rye:											
Vern, C. I. 73 (Secale cereale)	724±7	496±9	622±7		469±8	800±11	625±5	6	634±8	634±8	634±8
Native plants:											
Buffalo grass (Bulbilis dactyloides)			308±17					1	296±16	296±16	296±16
Grama grass (Bouteloua gracilis)											
Buffalo and grama grass mixed (Bulbilis dactyloides and Bouteloua gracilis)			389±12	389±7	312±14	336±8	290±9	4	338±11	338±11	
Clammyweed (Polanisia trachysperma)			502±11					1	374±12	374±12	
Iva (Iva xanthifolia)								1	483±11	483±11	
Western ragweed (Ambrosia elatior)			948±80			652±6		1	534±5	534±5	
Western wheat grass (Agropyron smithii)			1076±29					1	912±63	912±63	
Franseria (Franseria tenuifolia)			1131±50	1176±52				1	1055±28	c857±27	638±31
								1	1131±50	1181±50	
Cucurbits:											
Watermelon, Rocky Ford (Citrullus vulgaris)			600±15					1	577±14	577±14	577±14
Cantaloupe, Rocky Ford (Cucumis melo)			621±27					1	597±26		
Cucumber, Boston picking (Cucumis sativus)			713±11					1	686±11	642±21	
Squash, Hubbard (Cucurbita maxima)			748±8					1	719±8		
Pumpkin, common (Cucurbita pepo)			834±17					1	802±16	761±13	676±16
Rice:											
Honduras, C. I. 1643 (Oryza sativa)		519±13	744±17					2	682±17	682±17	682±17
Legumes:											
Guar (Cyamopsis)			571±3					5	523±8	523±8	
Cowpea, S. P. I. 29282 (Vigna sinensis)		510±14						5	569±6	569±6	
Chickpea, S. P. I. 24522 (Cicer arietinum)			805±8	544±8				2	638±18	638±18	
Clover, Crimson, S. P. I. 33742 (Trifolium incarnatum)			789±9	659±5	413±5	767±8	481±4	1	636±18		
Clover, Red, S. P. I. 34869 (Trifolium pratense)			682±4	517±19				1	750±9	698±14	
Bean, navy (Phaseolus vulgaris)			773±8					1	656±4		
Bean, Mexican (Phaseolus vulgaris)								1	743±8		
Vetch, Black Bitter (Vicia ervilia)			680±8	584±12				2	562±12	700±6	700±6
Vetch, hairy, S. P. I. 34298 (Vicia villosa)			772±11	531±5				1	587±6		
Bean, horse, S. P. I. 25645 (Vicia faba)			780±19					1	742±11		
Bean, horse, S. P. I. 15429 (Vicia faba)								1	750±18	708±12	
Vetch, purple, S. P. I. 18131 (Vicia atropurpurea)			935±9					1	899±9		
Bean, soy, cultivated, S. P. I. 21755 (Soja max)			672±9					1	646±9		
Bean, soy, wild, S. P. I. 25138 (Soja max)			815±25					2	784±24		
Clover, sweet, S. P. I. 21216 (Melilotus alba)	709±9	638±4						1	731±7	715±18	
Pea, Canada field, S. P. I. 30134 (Pisum sativum)								1	743±5	731±7	
Pea, Canada field, S. P. I. 22637 (Pisum sativum)								1	748±7	747±6	
Lupinus albus, S. P. I. 35477 (Lupinus albus)	800±7										
Alfalfa (Hansen seed) (Medicago falcata)			870±34					1	837±33	837±33	
Alfalfa, Peruvian, S. P. I. 30203 (Medicago sativa)			846±27					1	813±26		
			651±12					1	626±12		

a Not included in the mean.
b Average of genus Solanum.
c Average of genus Agropyron.

...yields during the years 1911–1917, inclusive—Continued

Plant	1911	1912	1913	1914	1915	1916	1917	Number of years for which record was made	Species or variety	Genus	Crop or group
Legumes—Continued.											
Alfalfa, Grimm, A. D. I. 162-98-A (Medicago sativa)				810±5	685±13			2	835±13		
Alfalfa, Grimm, A. D. I. E-23-20-52 (Medicago sativa)		657±11	834±8	908±11	695±9			3	831±11		
Alfalfa, Grimm, A. D. I. E-23 (Medicago sativa)				890±6		1047±9	822±8	4	866±9		
Alfalfa, Grimm, A. D. I. 162-98 (Medicago sativa)				904±12				1	869±12		
Alfalfa, Grimm, A. D. I. 162-98-B (Medicago sativa)				906±12				1	871±12		
Alfalfa, Grimm, A. D. I. E-5-30 (Medicago sativa)				933±11				1	897±11		
Alfalfa, Grimm, S. P. I. 25695 (Medicago sativa)	1008±16	659±6						2	911±12		
Alfalfa, Grimm, A. D. I. H-4-60 (Medicago sativa)				957±8				1	920±8	844±13	750±14
Flax:											
Damont, C. I. 3 (Linum usitatissimum)								1	624±13		
Kashgar, S. P. I. 37719, C. I. 50-1 (Linum usitatissimum)					569±4		605±13	2	703±5		
North Dakota Resistant No. 114, C. I. 13 (Linum usitatissimum)			905±25					1	752±13		
Reserve, C. I. 19 (Linum usitatissimum)					579±10	812±0		2	835±18		
Soddo White, S. P. I. 37086, Abyssinia, C. I. 36 (Linum usitatissimum)					615±7			1	812±5		
Smyrna, S. P. I. 36949 (Turkey), C. I. 30 (Linum usitatissimum)					625±4			1	861±21		
Jalaun, S. P. I. 36566 (India), C. I. 21 (Linum usitatissimum)					663±16	1098±10		2	893±14	783±14	783±14
Grasses:											
Wheat-grass, S. P. I. 19537 (Agropyron desertorum)			705±27		682±14			1	678±26	[c]857±27	828±26
Brome-grass, S. P. I. 29880 (Bromus inermis)			1016±26					1	977±25	977±25	

[c] Average of genus Agropyron.

TABLE 34.—*Actual water requirement, based on grain, seed or tuber production, for each year, for each variety, genus and crop or group of all plants grown at Akron, Colo., period, 1911–1917, inclusive*

Plant	1911	1912	1913	1914	1915	1916	1917	Number of years for which record was made	Species or variety	Genus	Crop or group
Proso:											
Black Voronezh, C. I. 16		425±4						1	531±5		
Tambov, S. D. 366, Akron 366–1–0		482±9						1	603±11	567±9	567±9
Millet:											
Kursk, S. P. I. 22420	923±40							1	863±37		
Kursk, S. P. I. 34771		483±11	985±99		665±24	1267±45		5	898±51		
Siberian, A. D. I. 3–4				1075±38				1	1117±49	959±46	959±46
Buckwheat	1037±33			1162±51				1	969±31	969±31	969±31
Sorghum:											
Sorgo—											
Dakota Amber, A. D. I. 341–10–A				898±50				1	863±48		
Minnesota Amber, A. D. I. 341–13			765±12	893±26				6	1043±96		
Red Amber, S. P. I. 17543	1494±202	2366±194	1100±31		1116±105	853±26	1700±224	3	1804±179	1237±120	
Grain sorghum—											
Blackhull Kafir, S. P. I. 24975	803±26							1	750±24		
White Durra, S. P. I. 24997	809±12		753±11					1	753±11		
Brown Kaoliang, S. P. I. 24993	726±12	927±38			919±35			2	919±35		
Dwarf Milo, S. P. I. 24970	1123±57							1	1050±53	868±34	1028±83
Barley:											
Beldi, C. I. 190	1155±18	941±10						2	1128±15		
Hannchen, C. I. 531	1394±27	1005±36		1179±28	949±50	1425±27	1148±12	6	1172±37		
Beardless, C. I. 716	1210±38	1017±83						2	1201±78		
Nepal, C. I. 595	1475±40	1239±11						2	1464±28	1241±46	1241±46
Corn:											
Indian Flint	2040±342	954±106	854±31					1	821±30		
Northwestern Dent			1241±77		2060±108	3634±19	1929±73	6	1999±199	1405±114	1405±114
Cucurbits:											
Watermelon, Rocky Ford			1146±49					1	1102±47	1102±47	
Cucumber, Boston pickling			1611±67					1	1549±64		
Cantaloupe, Rocky Ford			1824±237					1	1754±228	1652±167	1468±139
Crucifers:											
Turnip, purple top			1530±132					1	1471±127	1471±127	1471±127
Oats:											
Sixty-Day, C. I. 165	1383±30	1172±183		1483±31	1150±27	1975±49	1854±91	2	1379±119		
Burt, C. I. 293	1500±57	1224±55		1421±8	1102±34	2288±39	2088±37	7	1566±55		
Swedish Select, C. I. 134	1632±35	1103±18	1641±33					7	1648±36		
Canadian, C. I. 444	2204±140	1416±119	1876±55					2	1915±140	1627±97	1627±97
Wheat:											
Emmer, C. I. 2951	1180±42	984±18						2	1167±32	1167±32	
Durum—											
Kubanka, C. I. 1440	1191±14	1111±37	1322±16	1367±13	1232±13	1779±108	1367±21	7	1365±44		
Beloturka, C. I. 3705				1240±35	1122±11			2	1372±23		
Beloturka, C. I. 3705 (old seed)					1129±11			1			
Jumillo, C. I. 1736				1310±22	1179±15			2	1396±20		

Variety	(1)	(2)	(3)	(4)	(5)	(6)	(7)	No.	Average
Kubanka, C. I. 2094				1286±23	1042±28	1857±142		1	1522±116
C. I. 4131, S. P. I. 37159 (from Siberia)					944±15	2125±104		2	1573±77
C. I. 4131 (old seed)						2640±170		2	
C. I. 4131 (1st generation)						2095±104		1	
C. I. 4131 (2d generation)								1	
C. I. 4082, S. P. I. 36388 (from Peru)				1461±38	1315±28	2291±172		2	1622±73
C. I. 4082 (old seed)					1299±38	1903±80		2	1475±68
C. I. 4082 (1st generation)						1821±60		1	
C. I. 4082 (2d generation)								1	
Common—									
Turkey, C. I. 1571		995±22						1	1244±28
Kharkof, C. I. 1583	1382±43	1064±60						1	1330±75
Ghirka, C. I. 1517		1468±34						2	1564±42
Marquis, C. I. 3641				1469±14	1279±22			2	1691±91
Marquis, C. I. 3641 (old seed)					1190±15			1	
Marquis, C. I. 3641 (1st generation)								1	
Marquis, C. I. 3641 (2d generation)								1	
Galgalos, C. I. 2398	1245±13			1900±30	1551±65	2395±232	1516±19	5	1714±70
Marvel Bluestem, C. I. 3082	1786±60	1573±49				2339±126		2	1818±60
C. I. 4090, S. P. I. 36502 (Turkestan)				1631±26	1617±20	2008±43		2	1851±36
C. I. 4090 (old seed)					1451±39	2445±155		1	
Preston, C. I. 3328				1512±22	1788±71			1	1888±67
Haynes Bluestem, Minn., 169, C. I. 2874				2093±43	1766±39	2338±78		2	1916±64
C. I. 4637, S. P. I. 36499					1725±25			2	2182±42
C. I. 4087 (old seed)								2	
Pacific Bluestem, C. I. 4067				3564±98	3343±119	2959±346		3	3398±195
Hybrids—									
Kubanka, C. I. 2094×Haynes Bluestem, C. I. 2874				1987±30	1859±103	2431±103	1862±84	1	1995±84 / 2163±97
Jumillo, C. I. 1736×Prestor, C. I. 3328			2327±197		945±134	974±63	1144±17	2	1739±77
Potato:									
Irish Cobbler								3	1325±151
McCormick						3510±452		1	2877±370
Rye:									
Vern, C. I. 73	2215±37			2291±54	1485±80	2871±99			2142±76
Legumes:									
Cowpea, S. P. I. 29282	1802±62		1576±132	1962±27	1257±39	2373±77	1144±17	5	1632±41
Bean, navy			1646±86					1	1583±35
Bean, Mexican			1888±62					1	1815±60
Chickpea, S. P. I. 21322		1348±114						1	1685±143
Bean, soy, cultivate', S. P. I. 21755			2053±51					1	1974±49
Pea, Canada, field, S. P. I. 22637			2322±121					2	2153±105
Lupinus albus, S. P. I. 35477	2218±100			4923±478				1	4734±160 / 2225±190
Flax:									
Soddo, White, S. P. I. 37086, Abyssinia, C. I. 36					1548±17			1	2010±22
North Dakota Resistant No. 114, C. I. 13			2835±52		2054±87			1	2668±113
Reserve, C. I. 19					2388±57			2	2914±63
Jahun, S. P. I. 36566, Indian, C. I. 21					2420±101			1	3143±131
Smyrna, S. P. I. 36949, Turkey, C. I. 30					2785±82			1	3017±106
Kashgar, S. P. I. 37719, C. I. 50-1					3975±32			1	3162±42 / 3252±89

THE PROBABLE RANGE OF WATER REQUIREMENT OF DIFFERENT CROPS AT AKRON, COLO.

The weighted values in the last three columns of Tables 33 and 34 approximate closely the values for the period 1911 to 1917. A comparison of the evaporation rate for the period 1911–1917 with that of the longer period 1908–1925 shows that the means are very nearly the same and that no extremes exceed those recorded during the period 1911–1917. (See Table 32.) The writers feel justified, therefore, in concluding that the values of the water requirement given in Table 33 are essentially the values that would have been obtained had the experiment covered the full period 1908–1925 and therefore represent with considerable accuracy the water requirement of plants at this station. In Table 35 is given the weighted average value, the probable low values during damp cool years, and the probable high values during hot, dry years for the crops grown at Akron. The fourth column of the table under the heading "Probable extremes" indicates the basis on which these values have been obtained. The extremes serve as a correction of the more truly mathematical values given in column 3 under weighted mean. These are not to be regarded as measurements, but merely as the writers' deductions from the data in hand. They should serve as an expression of the probable results in the absence of actual measurements.

TABLE 35.—*Weighted mean water requirement based on dry matter, probable lowest and highest value, and units of dry matter produced for each 1,000 units of water consumed, based on the value of the weighted mean, for all plants grown at Akron, Colo., period 1911–1917, inclusive*

Plant	Number of years for which record was made	Weighted mean	Probable extremes of water requirement				Units of dry matter produced for every 1,000 units of water consumed (a)
			Lowest	Based on—	Highest	Based on—	
Proso:							
Black Voronezh, C. I. 15	1	258±1	206±1	Actual value 1912	346±1	Kursk millet, S. P. I 34771	3.88
Tambov, S. D. 366, Akron 366-1-0	1	260±1	208±1	do	348±1	do	3.85
Black Voronezh, S. D. 381	1	283±9	226±7	do	379±12 (?)	do	3.53
Millet:							
Kursk, S. P. I. 30029	1	216±13	173±10	do	280±17	do	4.63
Kursk, S. P. I. 34771 b	1	274±3	187±2	do	367±4	Actual value 1916	3.65
German, S. P. I. 26845	2	278±12	248±7	do	373±16	Kursk millet, S. P. I 34771	3.60
Siberian, A. D. I. 3-4	1	304±5	207±2	Kursk millet, S. P. I. 34771	407±7	do	3.29
Turkestan, S. P. I. 20694	1	368±8	250±5	do	493±11	do	2.72
Sorghum:							
Sorgo—							
Minnesota Amber, A. D. I. 341-13	6	274±3	239±2	Actual value, 1912	298±2	Actual value 1913	3.65
Dakota Amber, A. D. I. 341-10-A	1	285±1	214±1	Minnesota Amber	314±1	Minnesota Amber	3.51
Red Amber, S. P. I. 17543	3	287±3	215±2	do	316±3	do	3.48
Grain sorghum—							
Brown Kaoliang, S. P. I. 24993	2	282±2	212±2	do	310±2	do	3.55
Blackhull Kafir, S. P. I. 24975	2	292±6	219±5	do	321±7	do	3.2
White Durra, S. P. I. 24992	2	312±3	234±2	do	343±3	do	3.21
Milo, S. P. I. 24960	2	311±4	233±3	do	342±4	do	3.22
Dwarf Milo, S. P. I. 24970	2	324±4	243±3	do	356±4	do	3.09
Grass sorghum—							
Sudan Grass, S. P. I. 25017	5	350±3	266±3	Actual value 1915	426±3	Actual value 1916	2.63
Corn:							
Esperanza	2	299±5	212±4	Northwestern Dent	413±7	Northwestern Dent	3.34
Tom Thumb	1	303±8	215±6	do	418±11	do	3.30
Algeria	1	317±4	225±3	do	437±6	do	3.15
Indian Flint	1	329±5	234±4	do	454±7	do	3.04
Budapest	1	332±3	236±2	do	458±6	do	3.01
Hopi	1	347±9	246±6	do	479±12	do	2.88
Pima	1	351±7	249±5	do	484±10	do	2.85
Josquin	1	354±9	251±6	do	489±12	do	2.82
German C24-2	1	358±3	254±2	do	494±4	do	2.79
Northwestern Dent	1	361±10	253±7	Actual value 1915	495±13	Actual value 1916	2.77
Laguna	1	369±8	262±6	Northwestern Dent	509±11	Northwestern Dent	2.71
German C24-1	1	370±5	263±4	do	511±7	do	2.70

Variety	No.	Value 1	Value 2	Basis	Value 3	Basis	Relative
China White	3	375±7	266±5	do.	518±10	do.	2.67
Iowa Silvermine	2	396±7	274±5	do.	533±10	do.	2.59
Bloody Butcher	1	389±7	276±5	do.	537±10	do.	2.57
Hybrids—							
China White×Esperanza	1	313±3	222±2	do.	432±4	do.	3.19
China White×Hopi	1	332±3	236±2	do.	458±4	do.	3.01
Algeria×China	1	334±5	237±4	do.	461±7	do.	2.99
Joaquin×Budapest	1	351±5	249±4	do.	484±7	do.	2.85
German C24-1×2	1	358±1	254±1	do.	494±1	do.	2.79
China White×Laguna	1	361±5	256±4	do.	498±7	do.	2.77
Budapest×Pima	1	373±5	285±4	do.	515±7	do.	2.68
Joaquin×Pima	1	374±9	266±6	do.	516±12	do.	2.67
Teosinte:							
Durango	1	375±11	266±8	do.	518±15	do.	2.67
Hybrids—							
China White×Teosinte	1	362±4	257±3	do.	500±6	do.	2.76
Sugar beet:							
Morrison-grown, Kleinwanzleben	2	377±9	305±7	Kubanka wheat, C. I. 1440	494±12	Kubanka wheat, C. I. 1440	2.65
Weeds:							
Tumbleweed	2	260±6	198±5	Pigweed	294±7	Pigweed	3.85
Pigweed	6	305±6	229±2	Actual value, 1915	356±4	Actual value, 1911	3.28
Russian thistle	1	314±5	236±4	Pigweed	367±6	Pigweed	3.15
Lamb's-quarters	2	658±32	506±25	All crops	803±39	All crops	1.52
Polygonum	1	678±48	522±37	do.	827±59	do.	1.47
Barley:							
Beardless, C. I. 716	2	500±9	400±7	Hannchen, C. I. 531	658±12	Hannchen, C. I. 531	1.98
Beldi, C. I. 190	2	514±4	406±3	do.	668±5	do.	1.95
Hannchen, C. I. 531	6	523±8	404±11	Actual value, 1915	664±9	Actual value, 1916	1.91
Nepal, C. I. 595	2	528±2	417±2	Hannchen, C. I. 531	686±3	Hannchen, C. I. 531	1.89
Buckwheat	1	540±12	416±8	All crops	659±15	All crops	1.85
Wheat:							
Emmer, C. I. 2951	2	517±10	419±8	Kubanka wheat, C. I. 1440	677±13	Kubanka wheat, C. I. 1440	1.93
Durum—							
Beloturka, C. I. 3705, S. P. I. 35480	3	483±6	391±5	do.	633±8	do.	2.07
Kubanka, C. I. 1440		491±7	394±7	Actual value, 1912	636±14	Actual value, 1916	2.04
Jumillo, C. I. 1736	2	496±10	402±8	Kubanka wheat, C. I. 1440	650±13	Kubanka wheat, C. I. 1440	2.02
C. I. 4131, S. P. I. 3715 (from Siberia)	6	531±13	357±4	Actual value, 1915	719±15	Actual value, 1916	1.88
C. I. 4082, S. P. I. 36388 (from Peru)	7	548±12	413±6	do.	710±9	do.	1.82
Kubanka, C. I. 2094	1	570±25	462±20	Kubanka wheat, C. I. 1440	747±33	Kubanka wheat, C. I. 1440	1.75
Common—							
Turkey, C. I. 1571	1	455±8	369±6	do.	596±10	do.	2.20
Kharkof, C. I. 1583	3	456±8	369±6	do.	597±10	do.	2.19
Ghirka, C. I. 1617	1	529±38	428±31	do.	693±50	do.	1.89
Power, C. I. 3697	3	530±9	429±7	do.	694±12	do.	1.89
Marvel Bluestem, C. I. 3082	2	534±5	433±4	do.	700±7	do.	1.87
Preston, C. I. 3328	2	539±7	437±6	do.	700±9	do.	1.86
Glyndon, C. I. 2873	1	540±4	437±3	do.	707±5	do.	1.85
Marquis, C. I. 3641	6	550±8	412±3	Actual value, 1915	726±5	Actual value, 1915	1.82
Prelude, C. I. 4323	1	553±20	448±23	Kubanka wheat, C. I. 1440	724±38	Kubanka wheat, C. I. 1440	1.91
Galgalos, C. I. 2308	5	557±5	481±4	Actual value, 1915	652±8	Actual value, 1916	1.80
Pioneer	1	562	455	Kubanka wheat, C. I. 1440	736	Kubanka wheat, C. I. 1440	1.78

TABLE 35.—*Weighted mean water requirement based on dry matter, probable lowest and highest value, and units of dry matter produced for each 1,000 units of water consumed, based on the value of the weighted mean, for all plants grown at Akron, Colo., period 1911–1917, inclusive*—Continued

Plant	Number of years for which record was made	Weighted mean	Probable extremes of water requirement				Units of dry matter produced for every 1,000 units of water consumed (²)
			Lowest	Based on—	Highest	Based on—	
Wheat—Continued.							
Common—Continued.							
C. I. 400, S. P. I. 36502, Turkestan	3	584±6	473±5	Kubanka wheat, C. I. 1440	765±8	Kubanka wheat, C. I. 1440	1.71
Haynes Bluestem, Minn. 169, C. I. 2874	2	600±8	486±6	do	743±11	Actual value, 1916	1.67
Pacific Bluestem, C. I. 4067	3	619±5	491±4	Actual value, 1915	690±7	do	1.62
C. I. 4087, S. P. I. 36499	3	640±4	518±3	Kubanka wheat, C. I. 1440	838±5	Kubanka wheat, C. I. 1440	1.56
C. I. 4103	1	663±8	537±6	do	869±10	do	1.51
Hybrids—							
Kubanka×Haynes Bluestem	1	521±17	422±14	do	683±22	do	1.92
Jumillo×Preston	2	547±3	493±2	do	717±4	do	1.83
Cotton:							
Triumph	6	568±10	443±8	Actual value, 1915	657±11	Actual value, 1913	1.76
Potato:							
Irish Cobbler	4	499±11	329±4	do	659±15	do	2.00
McCormick	2	650±8	501±14	All crops	744±8	Actual value, 1916	1.54
Oats:							
Canadian, C. I. 444	2	539±11	370±8	Swedish Select, C. I. 134	772±6	Swedish Select, C. I. 134	1.89
Sixty-Day, C. I. 165	2	594±12	416±8	do	867±18	do	1.68
Swedish Select, C. I. 134	1	604±10	423±5	Actual value, 1912	876±21	Actual value, 1916	1.66
Burt, C. I. 293	1	606±5	445±5	Actual value, 1915	809±5	do	1.65
Native weeds:							
Purslane	1	281±11	214±8	Pigweed	318±12	Pigweed	3.56
Cocklebur	1	415±13	320±10	All crops	506±16	All crops	2.41
Nightshade	1	587±13	375±10	do	594±16	do	2.05
Buffalo Bur	1	536±7	413±5	do	654±9	do	1.87
Gumweed	2	585±23	450±18	do	714±28	do	1.71
Sunflower, annual	2	577±8	444±6	do	704±10	do	1.73
Sunflower, narrow-leaved from sand hills	1	548±11	422±8	do	669±13	do	1.82
Sunflower, narrow-leaved from heavy soil	1	744±19	573±15	do	908±23	do	1.34
Mountain sage	2	654±20	504±15	do	798±24	do	1.53
Verbena	1	702±19	541±15	do	856±23	do	1.42
Fetid marigold	1	847±25	652±19	do	1033±31	do	1.18
Crucifers:							
Cabbage, Early Jersey Wakefield	1	518±7	399±5	do	632±9	do	1.93
Turnip, purple top	1	614±30	473±23	do	749±37	do	1.63
Rape	1	714±7	550±5	do	871±9	do	1.04

Crop	No.			Actual value, 1915		Actual value, 1916	
Rye:							
Vern, C. I. 73	6	634±8	469±8	Grama grass, series 1914-1917	800±11	Grama grass, series 1914-1917	1.58
Native plants:							
Buffalo grass:	1	206±16	288±14	Actual value, 1917	346±19	Actual value, 1914	3.38
Grama grass	4	338±11	290±9	Grama grass, series 1914-1917	389±7	Grama grass, series 1914-1917	2.96
Buffalo and grama grass mixed	1	374±12	325±10	All crops	439±14	All crops	2.67
Clammyweed	1	483±11	372±8	do	580±13	do	2.07
Iva	1	534±5	411±4	do	651±6	do	1.87
Western ragweed	1	912±63	702±49	do	1113±77	do	1.10
Western wheat-grass	1	1035±28	797±22	do	1263±34	do	.97
Franseria	1	1131±50	871±39	do	1380±61	do	.88
Cucurbits:							
Watermelon, Rocky Ford	1	577±14	444±11	do	704±17	do	1.73
Cantaloupe, Rocky Ford	1	597±26	460±20	do	728±32	do	1.68
Cucumber, Boston pickling	1	686±11	528±8	do	837±13	do	1.46
Squash, Hubbard	1	719±8	554±6	do	877±10	do	1.39
Pumpkins, common	1	802±16	617±12	do	978±20	do	1.25
Rice:							
Honduras, C. I. 1643	2	682±17	525±13	do	832±21	do	1.47
Legumes:							
Guar	1	523±8	371±6	Cowpea	696±15	Cowpea	1.91
Cowpea, S. P. I. 29282	5	509±6	413±5	Actual value 1915	767±8	Actual value 1916	1.76
Chickpea, S. P. I. 24322	1	638±18	491±14	All crops	778±22	All crops	1.57
Clover, crimson, S. P. I. 33742	2	636±18	490±14	do	805±8	Actual value 1913	1.57
Clover, red, S. P. I. 34869	1	759±9	584±7	do	926±11	do	1.32
Bean, navy	1	650±4	505±3	do	800±5	do	1.52
Bean, Mexican	1	743±8	572±6	do	906±10	do	1.35
Vetch, Black Bitter	2	562±12	433±9	do	686±15	do	1.78
Vetch, hairy, S. P. I. 34298	1	587±6	452±5	do	716±7	do	1.70
Bean, horse, S. P. I. 25645	1	742±11	571±8	do	905±13	do	1.35
Bean, horse, S. P. I. 15429	1	750±18	578±14	do	915±22	do	1.33
Vetch, purple, S. P. I. 18131	1	899±9	692±7	do	1097±11	do	1.11
Bean, soy, cultivated, S. P. I. 21755	1	640±9	497±7	do	788±11	do	1.55
Bean, soy, wild, S. P. I. 25138	1	784±24	604±18	do	956±29	do	1.28
Pea, Canada field, S. P. I. 30134	1	745±5	574±4	do	909±6	do	1.34
Pea, Canada field, S. P. I. 22637	2	748±7	576±5	do	913±9	do	1.24
Clover, sweet, S. P. I. 21216	1	731±7	563±5	do	892±9	do	1.37
Lupinus alpus, S. P. I. 35477	1	837±33	644±25	do	1021±40	do	1.19
Alfalfa (Hansen seed)	1	813±26	626±20	do	992±32	do	1.23
Alfalfa, Peruvian, S. P. I. 30203	2	626±12	482±9	do	764±15	do	1.60
Alfalfa, Grimm, A. D. I. 162-98-A	3	835±13	668±10	Alfalfa, series 1912-1917	1060±17	Alfalfa, series 1912-1917	1.20
Alfalfa, Grimm, A. D. I. E-23-20-52	4	831±11	665±9	do	1055±14	do	1.20
Alfalfa, Grimm, A. D. I. E-23	1	866±9	693±7	do	1100±11	do	1.15
Alfalfa, Grimm, A. D. I. 162-98	1	869±12	695±10	do	1103±15	do	1.15
Alfalfa, Grimm, A. D. I. 162-98-B	1	871±12	697±10	do	1106±15	do	1.15
Alfalfa, Grimm, A. D. I. F-5-30	1	897±11	718±9	do	1139±14	do	1.11
Alfalfa, Grimm, S. P. I. 25695	1	911±12	729±10	do	1157±15	do	1.10
Alfalfa, Grimm, A. D. I. H-4-60	1	920±8	736±6	do	1168±10	do	1.09

TABLE 35.—*Weighted mean water requirement based on dry matter, probable lowest and highest value, and units of dry matter produced for each 1,000 units of water consumed, based on the value of the weighted mean, for all plants grown at Akron, Colo., period 1911–1917, inclusive—Continued*

Plant	Number of years for which record was made	Weighted mean	Probable extremes of water requirement				Units of dry matter produced for every 1,000 units of water consumed (a)
			Lowest	Based on—	Highest	Based on—	
Flax:							
Damont, C. I. 3	1	624±13	480±10	All crops	761±16	All crops	1.60
Kashgar, S. P. I. 37719, C. I. 50–1	2	703±5	541±4	do	858±6	do	1.42
North Dakota Resistant No. 114, C. I. 13	1	752±13	579±10	do	917±19	do	1.33
Reserve, C. I. 19	2	835±18	643±14	do	1019±22	do	1.20
Soddo White, S. P. I. 37086, Abyssinia, C. I. 36	1	812±5	625±4	do	991±6	do	1.23
Smyrna, S. P. I. 36949 (Turkey), C. I. 30	1	861±21	663±16	do	1050±26	do	1.16
Jabaun, S. P. I. 36566 (India), C. I. 21	2	893±14	688±11	do	1089±17	do	1.12
Grasses:							
Wheat-grass, S. P. I. 19537	1	678±26	522±20	do	827±32	do	1.47
Brome-grass, S. P. I. 29880	1	977±25	752±19	do	1192±31	do	1.02

a The term "productivity of transpiration" has been used to express the water requirement and is applied to the units of dry matter produced for every 1,000 units of water consumed, and is the reciprocal of the water-requirement multiplied by 1,000. See MAKSIMOV, N. A. (МАКSIMOV, N. A.) THE PHYSIOLOGICAL BASIS OF DROUGHT-RESISTANCE OF PLANTS. Trudy Prikl. Bot. i Selek. (Bul. Appl. Bot. and Plant Breeding) Sup. 26, 436 p., illus. 1926. [In Russian, English abstract, p. 398–407.]

b In 1911, S. P. I. 22420 was used.

THE PROBABLE ERROR OF THE WEIGHTED VALUES FOR WATER REQUIREMENT

The probable error expressed in these summary tables shows only the error due to experimentation or to the lack of uniformity of the six pots used in each set. Where several sets are averaged the probable error is also expressed for the average. In order to check these probable errors and to determine to what degree it is justifiable to weight the results, the whole series has been regarded as a single population and the value of each observation in the series has been raised or lowered by the proportionate amount. (See Table 36.) These weighted values have then been regarded as one population and their probable error estimated. The error of the weighted average includes only the experimental errors and does not take into account the variation of the weighted values during different years; while the error of the average of the weighted values includes in addition to the experimental errors the errors due to the weighting and the variations one year with another. A comparison of the errors of these averages shows that the error is not increased by including the errors due to the weighting of the values.

TABLE 36.—*Comparison of probable errors derived from weighted means of water-requirement measurements with probable errors derived from weighted individual determinations treated as a single population for crops grown at Akron, Colo., 1911–1917, inclusive*

Year	Alfalfa, weighted individual pots	Oats, Swedish Select, weighted individual pots	Oats, Burt, weighted individual pots	Barley, Hannchen, weighted individual pots	Wheat, Kubanka, weighted individual pots	Corn, Northwestern Dent, weighted individual pots	Millet, Kursk, weighted individual pots	Sorghum, Minnesota Amber, weighted individual pots
1911	1006	583	617	500	419	288	277	[a]271
	1000	564	591	445	479	325	263	282
	1007	615	621	499	426	356	267	267
	879	562	582	484	402	356	270	293
	1024	576	566	491	420	379	264	270
	1073	549	606	538	372	360	266	276
					446			
					464			
					489			
					438			
					460			
Weighted mean	998±15	575±7	597±7	493±7	437±7	344±9	268±2	[a]279±4
1912	905	520	544	573	440	429	240	284
	823	543	583	565	480	365	230	303
	790	523	566	553	491	358	228	295
	775	498	548	555	514	310	235	300
	784	544	570	545	523	316	228	303
	848	549	559	530	508	321	244	305
Weighted mean	821±14	529±6	561±4	554±4	493±0	350±13	234±3	299±3
1913	852	621	388		490	419	269	284
	831	631	592		478	350	275	298
	744	581	625		458	390	277	288
	770	568	390		493	434	269	289
	838	586	599		460	340	255	279
	789	573	563		483	361	303	284
	783							
	782							
	820							
	852							
	799							
	769							
Weighted mean	802±8	563±9	593±5		477±5	384±12	275±4	287±2

1914	897	570	604	462	483	372	277	288
	838	578	612	470	483	369	286	284
	861	578	605	493	520	352	279	268
	852	582	588	477	508	364	289	268
	846	567	571	478	488	349	283	269
	843	582	569	511	510	317	288	260
Weighted mean	856±6	576±2	591±6	482±5	498±6	354±6	284±2	273±3
1915	939	560	581	461	548	336	268	256
	891	538	582	492	547	273	261	286
	965	623	623	505	548	353	262	265
	857	638	579	578	506	322	255	270
	901	583	558	523	497	323	262	258
	866	551	547	588	506	366	275	249
Weighted mean	903±12	582±13	578±6	525±14	526±8	329±9	262±1	264±4
1916	848	752	652	580	570	380	309	253
	860	785	643	564	503	424	311	249
	832	746	665	537	466	393	302	244
	835	659	679	516	535	363	281	253
	880	721	678	539	548	430	307	235
	895	642	661	530	507	447	293	
Weighted mean	858±7	718±17	663±4	544±7	521±11	406±11	301±3	243±3
1917	802	639	682	527	604[a]	340	307	261
	837	639	666	522	494	362	287	300
	881	672	658	550	490	357	279	286
	840	654	658	542	474	373	312	271
	849	680	640	553	477	354	268	284
	874	641	630	535	472		304	280
Weighted mean	847±8	655±5	656±4	538±4	486±4	357±3	293±5	280±5
Weighted average (all values regarded as a single population)	861±6	604±7	606±4	523±4	485±4	361±4	274±2	275±2
Average of weighted means	869±11	604±10	606±5	523±8	491±7	361±10	274±3	275±4

[a] Red Amber, S. P. I. 17543.

SUMMARY OF WATER-REQUIREMENT MEASUREMENTS AT AKRON, COLO.

The results of the water-requirement measurements at Akron, Colo., from 1914 to 1917, inclusive, are here presented in detail. With these are summarized those previously published. Measurements totaled 288 sets of plants, or more than 1,800 pots, covering the period from 1911–1917, inclusive.

On the basis of the average year at Akron, Colo., it is possible to compare the widely different crops grown in this experiment with each other. The lowest values, based on total dry matter, were obtained for the millets, sorghums, and corns; the highest values for the native plants, flaxes, and legumes. The water-requirement range is very great, from 216 for Kursk millet to 1,131 for Franseria, a native weed. In other words, Franseria required five times as much water to produce a ton of dry matter as did Kursk millet.

If the water requirement of proso millet is taken as 1.00 the water-requirement values for the various crops in terms of proso would be: Millets 1.07, sorghum 1.14, corn 1.31, teosinte 1.40, sugar beet 1.41, weeds 1.66, emmer 1.94, barley 1.94, buckwheat 2.02, durum wheat 2.03, common wheat 2.09, crucifers 2.12, cotton 2.13, potato 2.15, native weeds 2.17, oats 2.18, rye 2.37, native plants 2.39, cucurbits 2.53, rice 2.55, legumes 2.81, flax 2.93, grasses 3.10. These are often large groups and there is great variation within the groups. In general terms, the millets, sorghums, and corns are most efficient. The small grains—barley, wheat, oats, and rye—required almost twice as much water, while the legumes required almost three times as much as the millets, sorghums, and corns. The lowest value obtained was for Kursk millet, S. P. I. 30029, 216, or on the basis of the comparison of groups given above a value of 0.81; the most efficient proso 258, or 0.97; the most efficient sorghum, Minnesota Amber, 274, or 1.03; the most efficient native weed, purslane, 281, or 1.05; the most efficient native plant, buffalo grass, 296, or 1.11; the most efficient corn, Esperanza, 299, or 1.12; the most efficient common wheat, Turkey, 455, or 1.70; the most efficient durum wheat, Beloturka, 483, or 1.81; the most efficient potato, Irish Cobbler, 499, or 1.87; the most efficient barley, Beardless, 506, or 1.90; the most efficient crucifer, cabbage, 518, or 1.94; the most efficient legume, guar, 523, or 1.96; the most efficient oats, Canadian, 529, or 1.98; the most efficient cucurbit, watermelon, 577, or 2.16; the most efficient flax, Damont, 624, or 2.34.

The most efficient plant is the introduced millet, Kursk, S. P. I. 30029, followed in order by Black Veronezh proso, C. I. 15; tumbleweed; Tambov proso; Kursk millet, S. P. I. 22420; Minnesota Amber sorghum; Kursk millet, S. P. I. 34771; German millet; purslane; brown kaoliang; Black Veronezh, S. D. 331, millet; Dakota Amber sorghum; Red Amber sorghum; Blackhull kafir; buffalo grass; and Esperanza corn, all of which have a water requirement under 300.

This list includes all prosos and sorgos and most of the millets, a few of the grain sorghums, and one each of native plants, native weeds, and corn. In other words, 16 of the 151 plants measured gave a water-requirement value based on dry matter below 300. Some of these belong to the group of plants showing most efficient use of water during hot, dry years, but others were relatively most efficient during the cool, damp years.

A list of the crops with water-requirement values ranging between 300 and 400 would include only the more inefficient millets, Siberian and Turkestan; three of the grain sorghums, White Durra, Milo, and Dwarf Milo; all of the corns except the most efficient, Esperanza; and sugar beet, pigweed, Russian thistle, and grama grass.

Crops with water-requirement values between 400 and 500 include only three of the most efficient durum wheats, Beloturka, Kubanka C. I. 1440, and Jumillo; the common wheats, Turkey and Kharkof; Irish Cobbler potato; cocklebur and nightshade among the weeds; and clammy weed, a native plant.

Crops with a water requirement between 500 and 600 include all the barleys; buckwheat; the three least efficient durum wheats, C. I. 4131 from Siberia, C. I. 4082 from Peru, and Kubanka C. I. 2094; most of the spring common wheats such as Ghirka, Power, Marvel Bluestem, Preston, Glyndon, Marquis, Prelude, Galgalos, Pioneer, and C. I. 4090 from Turkestan, and two wheat hybrids; two crucifers, cabbage and rape; cotton; three oat varieties, Canadian, Sixty-Day, and Swedish Select. Among the native weeds, buffalo bur, the annual sunflower, and one planting of narrow-leaved sunflowers from the sand hills, Iva, and gum weed; two cucurbits, watermelon and cantaloupe; four legumes, guar, cowpea, black bitter and hairy vetch.

The plants having water requirement values between 600 and 700 include two weeds, lamb's-quarters and polygonum; three common wheats, Haynes, Pacific Bluestem, and C. I. 4087; turnip; McCormick potato; Burt oats; mountain sage; rye; cucumber; rice; the legumes, chickpea, crimson clover, navy bean, and soy bean; the most efficient flax, Damont; and wheat grass.

The narrow-leaved sunflower and verbena, both native weeds; the Hubbard squash; the legumes, horsebean, Mexican bean, wild soy bean, Canada field pea, and sweet clover; the Kashgar and North Dakota Resistant flaxes are among the plants whose water requirement values range from 700 to 800.

Only a few plants have a water requirement as high as 800 to 900. They include a fall wheat, C. I. 4127, grown in the spring; fetid marigold, pumpkin, purple vetch, and all the alfalfas except two of the least efficient Grimms; and Reserve, Soddo White, Smyrna, and Jalaun flaxes.

Two varieties of Grimm alfalfa, S. P. I. 25695, and A. D. I. H–4–60, have a water requirement between 900 and 1,000. Within this range also are found brome grass and western ragweed. Western wheat grass exceeds 1,000, and *Franseria*, a weed, 1,100 in water requirement.

On the basis of unit production per 1,000 units of water required, values for the different crops range from 0.88 for Franseria to 4.64 for Kursk millet. The millets, sorghum, and the best of the corns, weeds, and native plants produce over 3 units of dry matter per 1,000 units of water; the corns, sugarbeet, the most efficient wheats, and some of the native weeds, and native plants produce 2 units of dry matter per 1,000 units of water; while the great majority of plants produce more than 1 but less than 2 units of dry matter per 1,000 units of water consumed.

The range in water requirement during different years at Akron is very great, the lowest values averaging about 60 per cent of the

highest. The greatest range is for oats, fôr which crop the lowest value is only 48 per cent of the highest value; while the lowest range is for sorghum, for which the lowest value is 80 per cent of the highest.

If the crops are so arranged that those having relatively the lowest water requirement during the cool, damp years are placed at the plus end of the scale and those having the lowest relative water requirement during the hot, dry years at the minus end of the scale, the values (+) or (−) being the difference of departures from the mean of all crops for the cool, damp year and the hot, dry year, they will rank as follows: Swedish Select oats +22, Northwestern Dent corn +21, cowpea +16, Burt oats +13, Kursk millet +13, Vern rye +7, Hannchen barley +5, Sudan grass +1, Kubanka wheat 0, alfalfa −5, pigweed −7, Minnesota Amber sorghum −10, Triumph cotton −13, Galgalos wheat −16, grama grass −38. The mean of all crops included is 0 and Kubanka wheat falls on this mean. If evaporation is treated as a crop its value is −4. If the plant is used as a potometer and the value is expressed as the water requirement, the value applies only to the particular plant used. If the water requirement obtained for a plant like oats were applied to a plant like grama grass to get the measure of seasonal difference in 1915 and 1916, granting that 1915 were known, the resulting error would have raised the value in 1916 from its proper figure of say 100, to 183. On the basis of evaporation from a water surface this error would have raised the value of grama grass for 1916 from 100 to 132. Based on the results of this study which cover only about 23 plants, the evaporation data falls near the mean for all plants and therefore probably represent a safer value for comparing year with year than the water requirement of a plant chosen at random. To apply the results obtained from any evaporation system or from any plant to another evaporation system or plant would almost surely lead to error.

If both water requirement and total production of dry matter of plants are measured during a dry, hot year and a cool, damp year their relative efficiency in use of water and production of dry matter may be easily shown. The values of combined units of departure from the mean of crops grown at Akron from 1914–1917, in terms of Akron climate, are as follows: Swedish Select oats, +88; barley, +57; Burt oats, +55; rye, +46; Kubanka wheat, +34; millet, +9; Galgalos wheat, +9; alfalfa, −1; pigweed, −15; cowpea, −18; Sudan grass, −27; sorghum, −46; corn, −48; grama grass, −55, and cotton, −73. The plus values indicate efficient use of water and production of dry matter during cool, damp years and inefficient use of water and production of dry matter during hot, dry years. The minus values show relative high production of dry matter and efficient use of water during dry, hot years and low production and inefficient use of water during damp, cool years.

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